Oxygen and sulphur dioxide additions to Sauvignon blanc: effect on must and wine composition

by

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Declaration

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SUMMARY

Sauvignon blanc wines have become increasingly popular in South Africa as it is a cultivar that can be easily manipulated in the vineyard and cellar to produce a range of wine styles. These wines are usually given aroma descriptors such as green pepper, grassy and asparagus; while other more tropical aromas include passion fruit and guava. These aromas are thought to be mainly caused by methoxypyrazines and volatile thiols. These compounds are known to be character impacting compounds of Sauvignon blanc and are present in the grapes in the aromatic form (methoxypyrazines) or as non-aromatic precursors (thiols) that can be released by the yeast during fermentation. Other aroma compounds such as esters, higher alcohols, fatty acids and monoterpenes are compounds that could potentially influence the aroma bouquet of a wine significantly. These aroma compounds exist either as precursors in the grapes (monoterpenes) or arise due to yeast metabolism during fermentation (esters, higher alcohols, fatty acids) and often display fruity, floral and pleasant aromas.

In the cellar, winemaking practices can be manipulated to a certain extent to achieve the desired wine style. Winemaking tools such as temperature, skin contact, pressing conditions, oxygen (O_2), sulphur dioxide (SO_2) and yeast strain are only a few factors influencing the outcome of a wine. In general, South African winemakers maintain a very reductive environment during Sauvignon blanc wine production by using inert gasses, thereby causing the production costs to increase. There is sufficient evidence to support the reductive handling of white wine, however there seems to be a lack of information as to why the must should be treated reductively before fermentation. The over all goal of this study was thus to investigate the effect of different O_2 and SO_2 additions to Sauvignon blanc must before settling, specifically focussing on the typical aroma compounds often found in these wines.

Chapter 2 gives an overview of the oxidation reactions occurring in must (enzymatic oxidation) and wine (chemical oxidation). This chapter also reports the origin of the specific Sauvignon blanc aroma compounds and their reaction to different must and wine treatments with a focus on oxidation. Chapter 3 reports research results focussing on the effect of the different must treatments on the character impacting compounds of Sauvignon blanc wines, specifically the methoxypyrazines and the volatile thiols. The effect of the treatments on the polyphenols and glutathione content in the must and wine was also investigated. Oxidation in the absence of SO_2 led to a decrease in glutathione and certain phenolic compounds in the must. In general, volatile thiols were protected against oxidation by SO_2, even when O_2 was present in the must. Methoxypyrazines concentrations were not significantly influenced by the treatments. Chapter 4 elucidates the effect of the treatments on other yeast and grape derived aroma compounds often found in Sauvignon blanc wines, such as the esters, higher alcohols, fatty acids and monoterpenes. In general, the effect of SO_2 seemed to have the greatest influence on the produced aroma compounds.

The results reported in this thesis could possibly change the way South African Sauvignon blanc musts are handled in future during the winemaking process. It is clear that O_2 and SO_2 management in the cellar is of critical importance for the winemaker to produce wines of high quality. Future work is important to fully understand the mechanisms and evolution of important aroma compounds of Sauvignon blanc wines during the winemaking process.

OPSOMMING

Sauvignon blanc wyn aroma word gewoonlik beskryf met terme soos groen rissie, grasagtig en aspersie terwyl ander tropiese aromas soos grenadella en koejawel ook dikwels voorkom. Die manipulasie van Sauvignon blanc in die wingerd en in die kelder tydens wynmaak, gee die wynprodusent die vryheid om 'n wye reeks wyn style te produseer. Dit maak Sauvignon blanc baie populêr in die Suid-Afrikaanse wynindustrie. Die bogenoemde aromas word waargeneem in die wyn as gevolg van die teenwoordigheid van sekere aroma komponente genaamd metoksipirasiene en vlugtige tiole. Hierdie komponente lewer 'n unieke bydrae tot die aroma samestelling van Sauvignon blanc wyne en kom voor in die druiwe in die aromatiese vorm (metoksipirasiene) of as nie-aromatiese voorlopers (tiole) wat tydens alkoholiese fermentasie deur die gis vrygestel kan word. Komponente soos esters, hoër alkohole, vetsure en monoterpene kan ook 'n potensiële bydra lewer tot die algehele aroma van Sauvignon blanc wyne en kom voor in die druiwe (monoterpene) of ontstaan as gevolg van gis metabolisme gedurende alkoholiese fermentasie (esters, hoër alkohole, vetsure). Hierdie geur komponente word dikwels beskryf as vrugtig, blomagtig en oor die algemeen aangenaam.

Tydens wynmaak kan die wyn tot 'n mate gemanipuleer word om 'n spesifieke wynstyl te bekom. Hulpmiddels soos temperatuur, dopkontak, pers omstandighede, suurstof (O_2), swawel dioksied (SO_2) en gisras is slegs 'n paar faktore wat die algemene uitkoms van 'n wyn kan beïnvloed. Oor die algemeen word Sauvignon blanc in Suid-Afrika baie reduktief behandel tydens wynbereiding. Dit vereis sekere hulpmiddels, soos die gebruik van inerte gas, wat die produksiekoste dikwels verhoog. Navorsing ondersteun die reduktiewe behandeling van wit wyn, maar dit wil voorkom asof daar 'n tekort aan navorsing is wat die reduktiewe behandeling van die sap voor fermentasie regverdig. Die algemene doel van die studie is dus om die effek van verskillende O_2 en SO_2 byvoegings tot Sauvignon blanc sap (voor afsak) te ondersoek met die fokus op die tipiese aroma komponente wat in die wyn voorkom.

Hoofstuk 2 lewer 'n algemene oorsig van die tipes oksidasie reaksies wat voorkom in sap (ensiematiese oksidasie) en wyn (chemiese oksidasie). Spesifieke Sauvignon blanc aroma komponente word ook ondersoek in terme van die oorsprong van die komponente asook die reaksie wat plaasvind met verskillende mos en wyn behandelings, met 'n fokus op oksidasie. In hoofstuk 3 word die effek van die verskillende mos behandelings op tipiese Sauvignon blanc aroma komponente, spesifiek metoksipirasiene en vlugtige tiole, ondersoek. Die effek van die behandelings op die polifenole en glutatioon inhoud in die mos en wyn word ook gerapporteer. Oksidasie van die sap in die afwesigheid van SO_2, het 'n afname in glutatioon en sekere polifenol konsentrasies veroorsaak. Dit wil voorkom asof die produksie van vlugtige tiole oor die algemeen beskerm word teen oksidasie indien daar genoegsame SO_2 teenwoordig is. Hierdie effek word ondervind selfs as die sap met suursof versadig word. Die effek van die behandelings op die konsentrasies van metoksipirasiene was nie beduidend nie. Hoofstuk 4

rapporteer die effek van die behandelings op ander aroma komponente soos esters, hoër alkohole, vetsure en monoterpene. Oor die algemeen wil dit voorkom asof die effek van SO_2 beduidend was en waarskynlik die grootste invloed op die produksie van hierdie aroma komponente het.

Na aanleiding van die resultate bevind in hierdie tesis, is daar ‘n moontlikheid dat die manier waarop Sauvignon blanc wyne geproduseer word in Suid-Afrika, moontlik kan verander in die toekoms. Vir die wynmaker om hoë kwaliteit Sauvignon blanc wyne te produseer, is O_2 en SO_2 bestuur in die kelder van kardinale belang. Verdere navorsing moet steeds gedoen word om die meganisme en evolusie van belangrike aroma komponente in Sauvignon blanc wyne tydens die wynmaakproses, ten volle te verstaan.

This thesis is dedicated to my family.
Hierdie tesis is aan my gesin opgedra.

Biographical sketch

Carien Coetzee was born on 31 January 1986 in Bellville. She went to DF Malan High School and after matriculating in 2004 enrolled for a BScAgric degree, majoring in Viticulture and Oenology at Stellenbosch University. She obtained her Bachelor degree *cum laude* in 2008. In 2009, she enrolled for a MScAgric degree in Oenology at the same University. She has spent 1 month at the University of Auckland in New Zealand in 2009 as part of her MSc studies.

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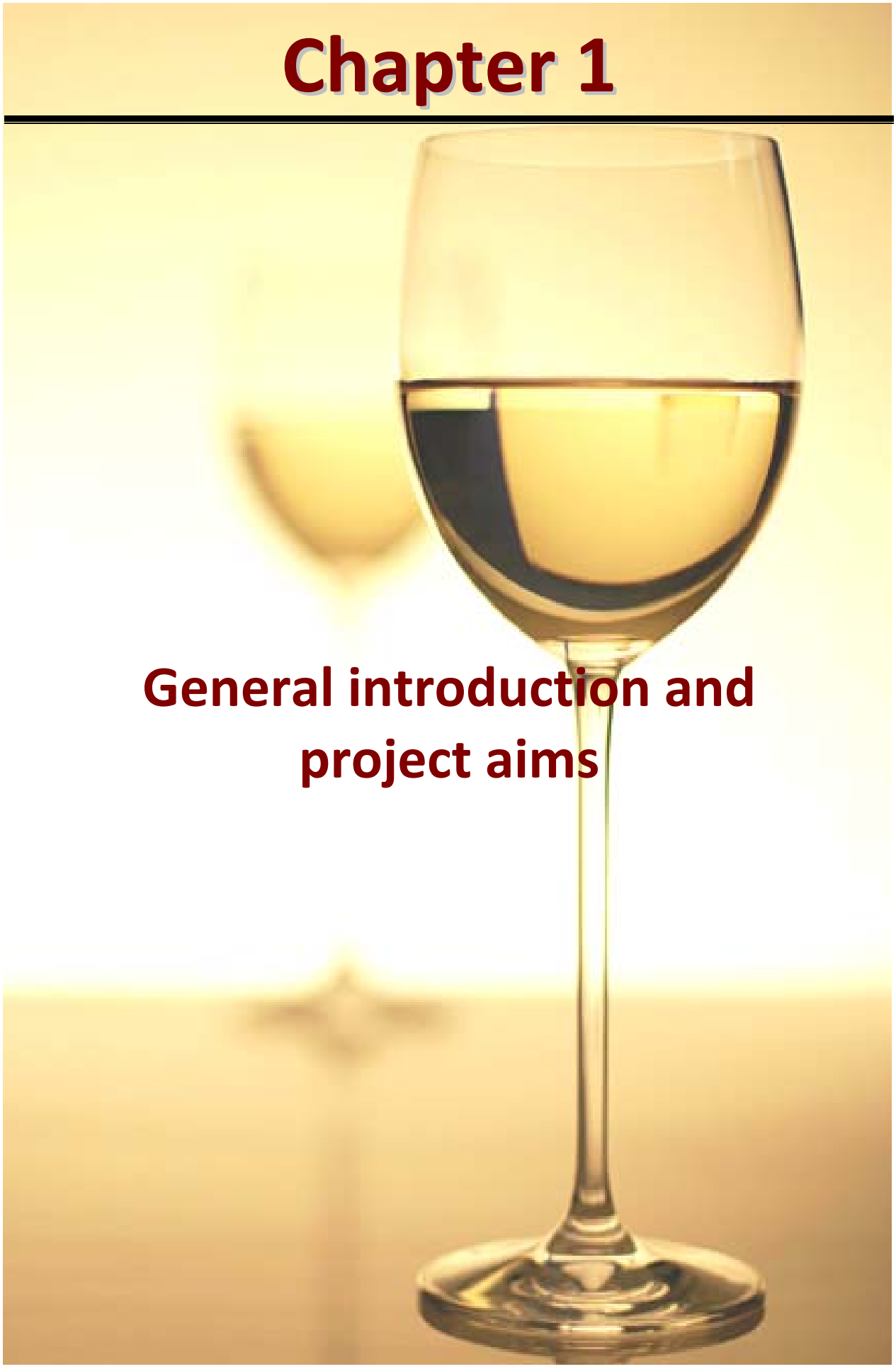
Preface

This thesis is presented as a compilation of 5 chapters. Each chapter is introduced separately. Chapters 3 and 4 are written according to the style of the *Journal of Agriculture and Food Chemistry* to which Chapter 3 is submitted for publication.

Chapter 1	**General introduction and project aims**
Chapter 2	**Literature review** Oxidation in white wines with a focus on Sauvignon blanc aroma
Chapter 3	**Research results** Effect of must oxygenation and sulphur dioxide addition on polyphenols, glutathione and certain aroma compounds in Sauvignon blanc
Chapter 4	**Research results** Effect of must oxygenation and sulphur dioxide addition on esters, higher alcohols, fatty acids and terpenes in Sauvignon blanc wine
Chapter 5	**General discussion and conclusions**

Contents

Chapter 1



General introduction and project aims

1. General introduction and project aims

1.1 INTRODUCTION

With a winemaking history dating back more than 300 years, the South African wine industry reflects the classicism of the Old World but is also influenced by the contemporary fruit-driven styles of the New World. In the last few years, a dynamic new vision has given momentum to changes within an industry which is innovation driven, market directed, globally competitive and highly profitable. With the advent of democracy, the opening of new markets and exposure to international trends, South Africa can now compete with confidence on the world wine stage. New wineries are opening up at a steady rate and are attracting increasing acclaim internationally. A passionate new generation of winemakers, many with experience of harvests around the globe, are keen to learn, experiment and consolidate. There's also been a focused shift from grape farming to wine growing. This all results in a greater variety of wine styles being produced, which not only increases the choice for the consumer, but also the competition between producers to place on the market a product at a lower production cost with a quality that is better and more consistent than their competitors.

Wine defects are considered to be unacceptable and should be identified rapidly and rectified if possible. During the winemaking process oxygen (O_2), can come into contact with the must and wine through various winemaking operations. It is only expected that the wine producer should want to know the reactions involved when O_2 comes into contact with the must and wine, especially the effect thereof on the aromatic profile of the bottled wine. In general, the exposure of the must to O_2 during winemaking, is considered to result in the disappearance or modification of aroma compounds. It has been found that wines made reductively had a more fruity character (Singleton *et al.*, 1980; Marais, 1998). Sauvignon blanc wines often have aromas reminiscent of green pepper, grass, guava, passion fruit etc. and play an important role in the portfolio of many South African wineries. South African winemakers often make use of aids such as inert gases, sulphur dioxide (SO_2) and ascorbic acid additions to protect Sauvignon blanc must from oxidation. These aids, especially the use of inert gases, can be extremely costly and winemakers go through great lengths to maintain an inert atmosphere during crushing and pressing. Oxidation processes and loss of certain aroma compounds in wine due to O_2 exposure, has been investigated extensively (Simpson, 1978; Marais *et al.*, 1992; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003; Blanchard *et al.*, 2004; Brajkovich *et al.*, 2005; Lopes *et al.*, 2009; Corona, 2010; Nikolantonaki *et al.*, 2010; Roland *et al.*, 2010). However, there seems to be a lack of information as to why Sauvignon blanc juice prior to fermentation needs to be treated reductively. This is especially important as it has been found that important aroma precursors such as the volatile thiol precursors in the juice are not susceptible to oxidation reactions (Roland *et al.*, 2010). This study is part of a wider

program at The Department of Viticulture and Oenology, Stellenbosch University, on the effects of O_2 in wine.

1.2 PROJECT AIMS

This study focussed on the effects of different O_2 and SO_2 additions to the juice on the chemical composition of Sauvignon blanc musts and wines. The main aims of this project were thus:

(i) To determine the effect of different combinations of O_2 and SO_2 additions to the must on the polyphenol and glutathione content in the must and wine;

(ii) To elucidate the effect of different combinations of O_2 and SO_2 additions to the must on the content of typical Sauvignon blanc aroma impact compounds such as the volatile thiols in the wine and methoxypyrazines in the must and wine;

(iii) To investigate the effect of different combinations of O_2 and SO_2 additions to the must on additional yeast derived aroma compounds such as esters, higher alcohols, fatty acids as well as terpenes originating from the grapes.

1.3 LITERATURE CITED

Blanchard, L., Darriet, P. & Dubourdieu, D., 2004. Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. Am. J. Enol. Vitic. 55, 115-120.

Brajkovich, M., Tibbits, N., Peron, G., Lund, C. M., Dykes, S. I., Kilmartin, P. A. & Nicolau, L., 2005. Effect of screwcap and cork closures on SO_2 levels and aromas in Sauvignon blanc wine. J.Agric. Food Chem. 53 (26), 10006-10011.

Corona, O., 2010. Wine-making with protection of must against oxidation in a warm, semi-arid terroir. S. Afr. J. Enol. Vitic. 31 (1), 58-63.

Escudero, A., Asencio, E., Cacho, J. & Ferreira, V., 2002. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chem. 77, 325-331.

Lopes, P., Silva, M. A., Pons, A., Tominaga, T., Lavigne, V., Saucier, C., Darriet, P., Teissedre, P. L. & Dubourdieu, D., 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. J. Agric. Food Chem. 57, 10261-10270.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. S. Afr. J. Enol. Vitic. 19 (1), 10-16.

Marais, J., Van Wyk, C. J. & Rapp, A., 1992. Effect of storage time, temperature and region on the levels of 1,1,6-trimethyl-1,2-dihydronaphthalene and other volatiles, and on quality of Weisser Riesling. S. Afr. J. Enol. Vitic. 13, 33-44.

Nikolantonaki, M., Chichuc, I., Teissedre., P. L. & Darriet, P., 2010. Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. Analytica Chimica Acta. 660, 102-109.

Roland, A., Vialaret, J., Razungles, A., Rigou, P. & Schneider, R., 2010. Evolution of *S*-Cysteinylated and *S*-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. J. Am. Chem. Soc. 58, 4406-4413.

Silva Ferreira, A. C., Hogg, T. & De Pinho, P. G., 2003. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J. Agric. Food Chem. 51.

Simpson, R. F., 1978. Aroma and compositional changes in wine with oxidation, storage and ageing. Vitis. 17 (274-287).

Singleton, V. L., Zaya, J., Trousdale, E. & Salgues, M., 1980. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. Am. J. Enol. Vitic. 31 (1), 14-20.

Chapter 2

Literature review

Oxidation in white wines with a focus on Sauvignon blanc aroma

2. OXIDATION IN WHITE WINES WITH A FOCUS ON SAUVIGNON BLANC AROMA

2.1 OXYGEN IN WINE

2.1.1 INTRODUCTION

Oxygen plays a very important role during the winemaking process. It can potentially influence the composition and quality of must and wine. The atmosphere consists of roughly 21% oxygen and wine can never be completely protected from it.

Generally, the addition of oxygen to white juice and wine is undesirable. Even small amounts of oxygen can lead to oxidation of juice and wine and can lead to a loss in varietal aroma, especially fruitiness, often causing the wine to become “flat”. The presence of oxygen leads to the disappearance or modification of aroma compounds. In general, wines made reductively had been found to have a more preserved fruity character as low additions of the antioxidant, sulphur dioxide to healthy grapes resulted in more fruity wines (Singleton *et al.*, 1980; Marais, 1998).

This review will focus on the basic steps involved in oxidation processes occurring mainly in white juice and wine. It would also focus on the aroma compounds often found in Sauvignon blanc grapes and wine and the effect oxidation has the aromatic composition of this wine.

2.1.2 BASIC OXIDATION REACTION

Oxidation is a process where electron transfer takes place between a reductive and oxidative molecule. An electron is removed from an atom, or a group of atoms and is transferred to an receiving atom (Fig 2.1). In juice and wine, oxygen is the most important oxidation agent.

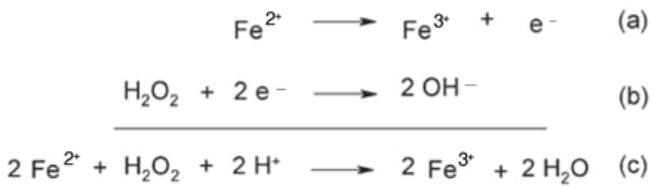


Figure 2.1 Redox system: oxidation of ferrous to ferric ion (a); reduction of hydrogen peroxide to hydroxide (b); and overall redox reaction (c).

2.1.3 DISSOLUTION AND CONSUMPTION OF OXYGEN IN JUICE AND WINE

2.1.3.1 THE RATE OF DISSOLUTION AND CONSUMPTION

The solubility of oxygen in juice and wine is influenced by solid particulate content, temperature, composition of the gas and ethanol concentration of the wine (Waterhouse & Laurie, 2006). The dissolution of oxygen can happen rapidly and can exceed levels of up to 2 mg/L/min (Ribéreau-Gayon *et al.,* 2006a). When juice is saturated with oxygen, it contains about 6-8 mg/L oxygen at cellar temperatures (Singleton, 1987; Du Toit *et al.*, 2006). Temperature plays a large role in the dissolution process as O_2 dissolves more easily at lower temperatures. Between 5 ^{O}C and 35 ^{O}C the amount of oxygen required to saturate juice drops from 10.5 mg/L to 5.6 mg/L (Ribéreau-Gayon *et al.,* 2006b).

The consumption of the oxygen by the juice (oxidation reaction occurs) is determined by a few factors such as available substrates (phenolic concentrations) temperature, enzymatic activity, and the competition between different substances for binding (White & Ough, 1973). The oxidation reaction is accelerated at elevated temperatures, with the consumption rate normally being up to three times faster at 30 ^{O}C than at 12 ^{O}C (Dubernet & Ribéreau-Gayon, 1974). In wine, the consumption rate is slower due to the lack of catalytic oxidation enzymes (Ribéreau-Gayon *et al.*, 2006a).

2.1.3.2 WINEMAKING PRACTICES THAT AFFECT OXYGEN DISSOLUTION AND CONSUMPTION IN WINE

Winemakers in South Africa often use antioxidants such as SO_2 and ascorbic acid to protect the juice and wine from oxidation. The use of inert gases such as carbon dioxide and nitrogen are also employed to replace the air in contact with the medium. Even though great care is taken the opinion that the juice needs to be treated reductively, is not unanimous. In general, the aim of reductive handling is to conserve the green colour of the juice and transfer this reductive state to the wine to preserve fruity aromas (Singleton *et al.*, 1980; Schneider, 1998). However, treating a wine too reductively may increase its potential to oxidise rapidly when coming in contact with oxygen at a later stage. Hyperoxidation, where the substrates for oxidation are removed in the juice by forced oxidation, can thus have a positive influence on the oxidative stability and aromatic composition of a wine (Mauricio *et al.,* 1997; Valero *et al.,* 2002; Roland *et al.,* 2010b).

Table 2.1 shows different winemaking practices that could lead to significant oxygen dissolution. Major oxidation can occur during pressing, depending on the kind of press used and pressing management (Cheynier, 1993). During racking, oxygen can dissolve in the juice in the range of 2-5 mg/L. Further practices like filtration, pumping and centrifugation (practices performed with vigorous agitation in open air) can lead to oxygen saturation in the juice, although the amount of oxygen dissolved is

dependant on the technique used and the care taken to exclude oxygen. These practices can have great implications if not applied correctly, especially with the handling of juice, where rapid oxidation can take place due to the presence of oxidation enzymes (Ribéreau-Gayon *et al.*, 2006b).

Table 2.1 Winemaking practices and their potential oxygen uptake

Winemaking practice	Potential amount of oxygen dissolved
pressing	10-15 mg/L (pressing of whole clusters)
pumping	2 mg/L
transfer from tank to tank	up to 6 mg/L
filtration	4-7 mg/L
racking	3-5 mg/L
centrifugation	up to 8 mg/L
bottling	0.5-3 mg/L
barrel aging	20-45 mg/L/year

(Cheynier, 1993; Du Toit *et al.*, 2006)

2.1.3.3 BUFFER CAPACITY FOR OXIDATION

Wines have a certain buffer capacity for oxidation. This buffer capacity is determined by the concentration of oxidizing or reducing agents in the medium. Red wines typically have a high buffer capacity as they have a high phenol content. White wines have a much lower phenolic content as they are produced in such a manner to avoid high phenolic extraction. This lower buffer capacity renders white wine more vulnerable to oxidation. In the case of oxygen exposure, red wine will oxidize more rapidly, as there are more phenols to serve as substrates, but the wine will rapidly recover because of its high buffer capacity. White wines, on the other hand, will undergo a slower oxidation (less substrate), but will not be able to recover to the same extent (Ribéreau-Gayon *et al.*, 2006b). The total phenol content could thus be a measurement of wine's capacity to take up oxygen, withstand oxidation and its capacity to change when coming in contact with oxygen (Singleton, 1987).

2.1.4 OXIDATION REACTIONS IN GRAPE MUST

In juice, oxidation enzymes are present and the main form of oxidation that takes place is enzymatic oxidation, which occurs at a much faster rate than chemical oxidation. In healthy grapes polyphenol oxidase (PPO) enzymes occur, whereas laccase originates from *Botrytis cinerea* infected grapes. These oxidation enzymes catalyse oxidation reactions in juice and must (Dubernet & Ribéreau-Gayon, 1973; Dubernet & Ribéreau-Gayon, 1974). Oxidation enzymes can be inhibited by the addition of SO_2. Sulphur dioxide can inhibit and destroy PPO; total activity decreases 75% to 90% when 50 mg/L SO_2 are added

(Dubernet & Ribéreau-Gayon, 1974). Laccase is more difficult to deactivate than PPO and are able to consume oxygen over longer periods of time and at faster rates (Peynaud, 1984). Laccase also has a wider substrate spectrum. Care should thus be taken when making wines from grapes infected by *Botrytis cinerea* and higher levels of SO_2 will be needed to inhibit laccase.

During the winemaking process, the oxidation enzymes normally lose their activity and precipitate and are usually deactivated by the time fermentation is completed (Ribéreau-Gayon *et al.*, 2006b). However, wine can still be oxidised in the presence of air by means of chemical oxidation.

2.1.4.1 SUBSTRATES FOR OXIDATION IN JUICE

Phenols originate from the grapes and total phenol concentrations range from 50-250 mg/L in white wines, which is less than 10 % of those found in red wines. This concentration can vary according to the cultivar and winemaking techniques used. White juice that has received prolonged skin contact will have higher concentrations of these compounds (Margalit, 1997; Monagas *et al.*, 2005). Phenols are the main compounds responsible for the browning of the white grape must (Cheynier, 1995).

In white wine and juice, non-flavonoids, such as hydroxybenzoic and hydroxycinnamic derivatives, normally constitute the main phenolic compounds. These derivatives consist mainly of the tartaric esters of *trans*-*p*-coumaric acid, *trans*-caffeic acid (the main hydroxycinnamic acid in wine) and traces of *trans*-ferulic acid (Ribéreau-Gayon, 1965). *Trans*-caftaric and coutaric acids occur at high concentrations in the liquid part of the grapes and are the main phenolics in white wine that did not receive prolonged skin contact (Betés-Saura *et al.*, 1996; Margalit, 1997; Monagas *et al.*, 2005). *Trans*-caftaric acid has been found to be present in Sauvignon blanc juice in the range of 113-178 mg/L, coutaric acid ranged from 21-68 mg/L and fertaric acid has been found to be present at very low concentrations in Sauvignon blanc juice (Vrhovšek, 1998).

These compounds have been identified as oxidation substrates and browning precursors in white wines (Singleton *et al.*, 1984; Cilliers & Singleton, 1989). The main substrates for oxidation enzymes are *trans*-caftaric and coutaric acid (Singleton *et al.*, 1984; Cheynier, 1989). However, coutaric acid appears less susceptible to oxidation degradation (Patel *et al.,* 2010).

Other phenols present are the flavan-3-ols namely (+)-catechin, (-)-epicatechin, procyanidins (B1 and B2) and flavonols such as quercitin, which normally occur in white wine at 1-3 mg/L (Ribéreau-Gayon, 1964; Weinges & Piretti, 1972). The flavanoid based phenolics (mainly (+)-catechin) are mainly found in the skins, stems and pips.

It is mostly the di- and trihydroxyphenols which oxidise easily under wine conditions. The most susceptible to oxidation are those containing an *o*-diphenol (catechol) functional group. It appears that electron withdrawing substitutes, like carboxyl or carboxyester groups, decrease the substrate's ease of

oxidation. Electron supplying substitutes, like methyl or hydroxyl substituents, would generally increase the oxidizability (Singleton, 1987).

Another substrate is ascorbic acid, which occurs naturally in grapes or can be added to the juice. This compound is usually absent by the end of must preparation (Singleton, 1987). Sulphur dioxide could be a consumer of oxygen, but under must and wine conditions the main free form, HSO_3^-, does not readily react with oxygen (Singleton, 1987). Acids such as tartaric, malic and lactic acids can be oxidised, but modifications are minimal (Singleton, 1987; Ribéreau-Gayon *et al.*, 2006b). Metals such as iron and copper can react and complex with oxygen, but normally occur at very low concentrations in must and wine and rather serve as a catalyst in oxidation reactions (Singleton, 1987).

2.1.4.2 ENZYMATIC OXIDATION

Once grape berries are crushed, phenolic substrates are released into the must together with grape polyphenol oxidase (PPO) that, in the presence of oxygen, induces the enzymatic oxidation (Macheix *et al.,* 1991). As mentioned earlier, the substrates for PPO are almost exclusively hydroxycinnamic acids and their tartaric acid esters, *trans*-caftaric and coutaric acid. They are the first substrates to be oxidised by the natural PPO enzyme. The oxidation of these phenols (e.g. *trans*-caftaric acid) by the PPO enzyme transforms them into *o*-quinones (Fig 2.2) (Singleton, 1987).

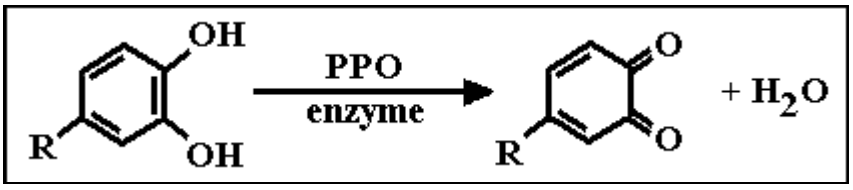


Figure 2.2 Mechanism for the enzymatic oxidation of a *o*-diphenol in the presence of oxygen and PPO enzyme (Hornsey, 2007)

The formed *o*-quinone is unstable and very reactive and can further partake in other reactions. *o*-Quinones are electrophiles and can readily react with nucleophilic centres such as other phenolic molecules, SO_2 and thiol containing compounds such as glutathione. The *trans*-caftaric acid *o*-quinone can enter into coupled oxidation reactions with flavonoids (flavanols) and it can condense with other phenolic compounds to form polymerized adducts (Cheynier *et al.,* 1988; Cheynier *et al.,* 1990). The formed adducts have low molecular weight and are not coloured compounds. An increase in the condensation degree will finally lead to the formation of yellow to brown pigments in must (Singleton, 1987). The formation of *o*-quinones is thus enzymatically driven, but the further reaction leading to browning, is non-enzymatic (Schneider, 1998). The colour of a young white juice is usually a light yellow and even a slight green tint. Juice that has undergone oxidation will develop a dark yellow to brown

colour due to the polymerization reaction. (Ribéreau-Gayon *et al.*, 2006a). After oxidative browning, the phenols can precipitate, leaving the resulting wines more colour stable (Singleton, 1987).

Other compounds such as polysaccharides and protein compounds can also affect the colour of white wines, but in the case of oxidised wines, phenolic compounds are mainly responsible for the colour change (Ribéreau-Gayon *et al.*, 2006b).

The consumption speed of the oxygen in juice is dependant on the activity, type and concentration of the oxidation enzyme, the amount of substrates (*trans*-caftaric acid) present and the presence of reducing agents such as SO_2, glutathione and ascorbic acid. The "trapping" of the *o*-quinone will prevent further addition and polymerization reactions from occurring. Temperature can also have an important effect as the rate of *o*-quinone formation is higher at higher temperatures (Zoecklein *et al.*, 1995).

2.1.5 OXIDATION REACTIONS IN WINE

2.1.5.1 DIRECT OXIDATION OF PHENOLS

At high pH conditions, phenols can react directly with oxygen. Phenolic compounds have a pK_a of 9 to 10 which allows them to form the phenolate anion that can react directly with oxygen. Wine and juice is acidic (pH 3 to 4) which limits this direct oxidation pathway (Singleton, 1987; Danilewicz, 2003).

2.1.5.2 THE ROLE OF METALS AND THE REDUCTIVE LADDER OF OXIDATION

Metals (iron and copper) serve as catalysts or initiators of oxidation reactions. Direct interaction of phenolics and oxygen does not occur unless catalyzed by transition metal ions (Danilewicz, 2003; Ribéreau-Gayon *et al.*, 2006b). The oxidizing potential of molecular oxygen in wine can be shown by the generation of reactive oxygen species (ROS) that constitute a stepwise ladder that indicate the reductive reaction taking place (Fig 2.3). While this reduction of the oxygen molecule is taking place, the oxidative reaction leads to the formation of an *o*-quinone from the phenolic substrate.

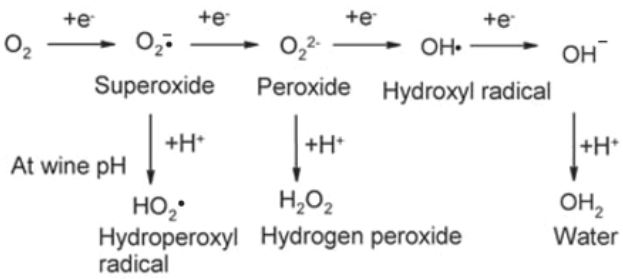


Figure 2.3 Reduction ladder of oxygen (Waterhouse & Laurie, 2006)

The initial transfer of an electron leads to the formation of the superoxide ion. At wine pH this ion exists as a hydroperoxyl radical. This step requires the presence of a catalyst presumably a transition state metal such as iron. The transfer of a second electron would yield a peroxide (hydrogen peroxide at wine pH). The next reduction step generates a very reactive hydroxyl radical. This radical is produced by the Fenton reaction between hydrogen peroxide and ferrous iron salts, generating water in the process (Green & Hill, 1984; Danilewicz, 2003; Waterhouse & Laurie, 2006).

2.1.5.3 PRIMARY OXIDATION PRODUCTS IN WINE

When phenolics react with ROS, the reaction rate is dependant on its ability to form a stable product radical. Compounds such as 1,2,3-trihydroxyl group (pyrogallol) and 1,2-dihydroxy aromatic ring (catechol) are easiest to oxidise because the resulting phenoxyl semiquinone radical can be stabilized by a second oxygen atom. Wine phenolics that fall in this class of easily oxidisable substrates include caffeic acid, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, gallic acid, the procyanidins, hydrolyzable tannins and quercitin.

The catechol group appears to be the primary reacting species with hydroperoxyl radical. During the reaction, they form a semiquinone radical and hydrogen peroxide (Fig 2.4 reaction 2). The semiquinone radical then yields the *o*-quinone (Fig 2.4 reaction 3) and the hydrogen peroxide reacts with ferrous iron to yield hydroxyl radical (Fig 2.4 reaction 4) .

This hydroxyl radical is non selective and very reactive and can react with many wine components. Compounds present at high concentrations will more likely undergo oxidation by the radical. Ethanol is present in abundance and the main expected product would be acetaldehyde or keto acids from the oxidation of organic acids (Fig 2.4 reaction 5). Many other compounds can also be oxidised by this mechanism, particularly products of abundance in the wine. The expected products would be mostly ketones and aldehydes (Fenton, 1894; Wilderandt & Singleton, 1974; Singleton, 1987; Boulton, 2003; Danilewicz, 2003; Waterhouse & Laurie, 2006).

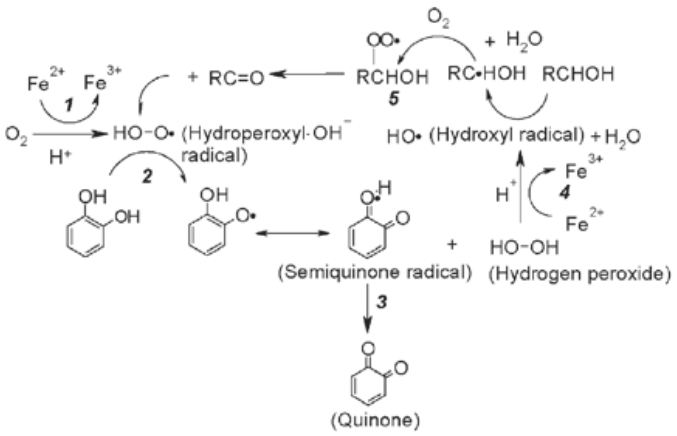


Figure 2.4 Primary oxidation products of some wine components

(Waterhouse & Laurie, 2006)

2.1.6 WINEMAKING PRACTICES TO PROTECT MUST FROM ENZYMATIC OXIDATION

Low temperatures in must delay oxidation reactions by lowering the enzyme's oxidation speed, but also leads to a higher O_2 dissolution capacity. Heating of must can denature the oxidation enzyme, rendering it unable to govern oxidation reactions. This action must be performed rapidly as it can have an inverted effect by accelerating oxidation reactions if the enzyme is not inhibited fast enough. This technique is not advised as temperatures of 45 ^{o}C and 65 ^{o}C are required to inactivate the PPO and laccase respectively (Ribéreau-Gayon *et al.*, 2006b).

Winemakers also use inert gasses such as carbon dioxide, nitrogen and even argon to prevent oxygen exposure. These gases are used to displace the air in pipes, presses, tanks or barrels and also cover the juice and wine with a protective gas blanket.

Clarification is also an effective method to reduce the amount of enzymes present, due to oxidation enzymes being associated with solids. Clarification is also a means of eliminating oxidation products like condensed flavonoids. Fining with bentonite reduces the soluble fraction of tyrosinase and a 30% loss in enzyme activity has been found with 100 g/hl bentonite addition (Ribéreau-Gayon *et al.*, 2006a). Phenolic molecules can also be removed by PVPP, gelatine and activated charcoal (Du Toit, 2003). Winemakers also limit the amount of phenolic substrates by removal of stems, soft pressing and minimizing skin contact.

Hyperoxidation (addition of large quantities of oxygen) can be used to remove oxidation substrates by oxidising the phenolic molecules which then precipitates. The formed pigments are insoluble in must, but more soluble in ethanol. It is therefore important to clarify the juice from the precipitate before the formation of ethanol during fermentation (Schneider, 1998).

2.1.6.1 THE USE OF SO_2 AND ASCORBIC ACID TO PREVENT ENZYMATIC OXIDATION IN MUST

Phenolic compounds and glutathione are antioxidants naturally present in grape must. Additional antioxidants, such as ascorbic acid and SO_2 can be added during winemaking. Sulphur dioxide has an antioxidant, antioxidasic and antimicrobial function and is added to must primarily to inhibit and potentially destroy the oxidation enzymes. It inhibits the enzymatic oxidation of phenols, sugars and amino acids, thus preventing browning of grape must (Haisman, 1974). The higher the concentration of added SO_2, the more effective it will be in inhibiting the enzyme. In order to destroy PPO enzymes, 50 mg/L of sulphur dioxide must be added to the juice (Ribéreau-Gayon *et al.*, 2006a). However, higher SO_2 concentrations are needed to inhibit the laccase enzyme.

According to Sayavedra-Soto and Montgomery (1986), it seems as though the bisulfite (HSO_3^-) form is responsible for this protection. Bisulfite binds via an irreversible reaction resulting in a structural modification rather than a binding product (Dubernet & Ribéreau-Gayon, 1973; Amano *et al.*, 1979; Sayavedra-Soto & Montgomery, 1986).

In the absence of SO_2, oxygen consumption is generally rapid, with levels in excess of 2 mg O_2/L/min in SO_2-free juice. Consumption of the dissolved O_2 can be complete in 4-20 minutes in a saturated white grape must. SO_2 retards oxygen consumption (Fig 2.5) and the rate of consumption will decrease over a period of time. The consumption rate will then stop and the dissolved oxygen concentration will stay stable, possibly leaving more dissolved O_2 available for yeast (Dubernet & Ribéreau-Gayon, 1974). A delay in the working of SO_2 could occur. It is therefore important that the grapes are healthy and SO_2 additions occur as soon as possible to prevent rapid enzymatic oxidation.

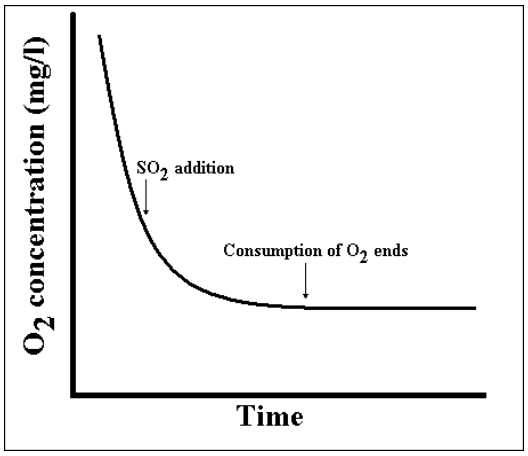


Figure 2.5 Oxygen consumption in musts following sulphiting (Dubernet & Ribéreau-Gayon, 1974)

Ascorbic acid has a powerful antioxidant property; however it does not have an inhibitory effect on PPO. When ascorbic acid is added to the must, it can protect phenols against oxidation by scavenging oxygen directly or by converting the *o*-quinone back to its original state in a coupled reaction. The *o*-quinone is reduced back to the phenol by the ascorbic acid, while the ascorbic acid is oxidised to dehydroascorbic acid, which is known to be relatively stable. It would thus only limit the browning of the must and not oxygen consumption by the oxidation enzyme. When using ascorbic acid during winemaking, oxygen should still be excluded as much as possible. In this coupled reaction, a strong oxidant, hydrogen peroxide, is formed. Free SO_2, in the form of HSO_3^- reacts with the peroxide forming sulfate and eliminates the oxidation potential of the peroxide. However, new evidence has come to the fore that ascorbic acid may act as a pro-oxidant when its concentration decreases and in the absence of SO_2 (Peng *et al.*, 1998; Marshall *et al.*, 2000; Bradshaw *et al.*, 2001; Danilewicz, 2003).

2.1.7 PREVENTION OF CHEMICAL OXIDATION AND THE ROLE OF SO_2

In wine, oxidation enzymes are inactivated and the primary oxidation that takes place is chemical oxidation. Chemical oxidation is slower than enzymatic oxidation. The general effects chemical oxidations have in white wine are the development of brown colour, production of aldehydes and the loss of varietal aroma.

A study done by Danilewicz *et al*., 2008, has provided evidence that the interaction of SO_2 with oxygen is quite complex (Fig 2.6) and involves a metal-catalysed radical chain reaction. Catechols are known to block this reaction. The scavenging effect of catechols involves reacting with the intermediate peroxomonosulfate radicals and so prevents the radical chain propagation. The direct interaction of oxygen and bisulfite is therefore unlikely to occur to a significant degree in wine, because of the scavenging activity of polyphenols (Danilewicz *et al.*, 2008).

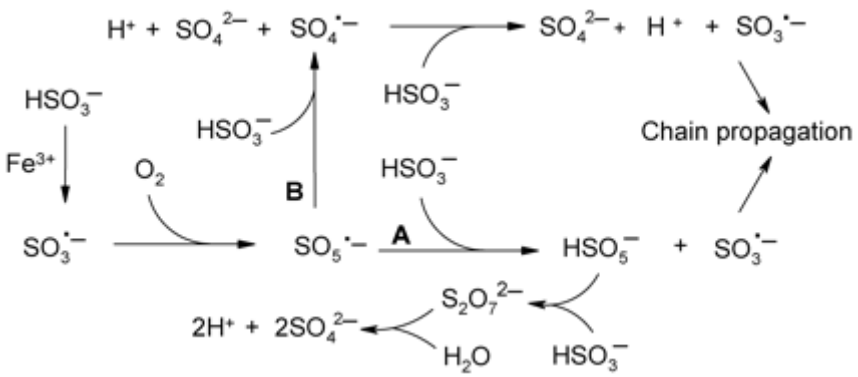


Figure 2.6 Radical chain reaction involved in bisulfite oxidation (Danilewicz *et al.*, 2008)

The antioxidant capability of SO_2 is mainly because of its ability to react with oxidants and/or be preferentially oxidised over other wine compounds. One of these oxidants can be hydrogen peroxide, which is a very strong and active oxidising agent. It is one of the products from the chemical oxidation of catechols (Fig 2.7) (Wilderandt & Singleton, 1974) and even ascorbic acid. In the absence of SO_2, it will continue to oxidise other wine compounds. Bisulfite is known to react directly and rapidly with hydrogen peroxide. This effect is limited as it has been shown, that even in the presence of 177 mg/L of sulphur dioxide, acetaldehyde was still formed (Wilderandt & Singleton, 1974).

Bisulfite is also capable of reacting with the *o*-quinone. Studies have been done with 1,4-benzoquinone and it has been established that half of the *o*-quinone was reduced back to 1,4-benzenediol (accompanied by the oxidation of sulfite to sulfate) and half underwent Micheal-type 1,4-addition to give sulphonic acid (Lu Valle, 1952; Youngblood, 1986; Danilewicz *et al.*, 2008). This mechanism is shown in Fig 2.7 and uses catechol as an example:

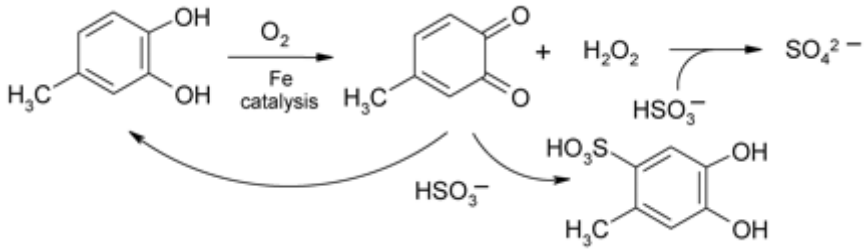


Figure 2.7 The interaction of bisulfite with hydrogen peroxide and *o*-quinones following catechol oxidation (Wilderandt & Singleton, 1974; Danilewicz *et al.*, 2008)

Sulphur dioxide reacts thus with the *o*-quinone and with the hydrogen peroxide. In wine, nucleophiles (such as polyphenols and thiols) are present and can compete with the bisulfites for *o*-quinones. It is thus critical to reduce the *o*-quinone back to the phenol. The reaction of SO_2 with the formed *o*-quinone is rapid. This reaction has been shown to be more rapid with caffeic acid when compared to the falvonoids (+)-catechin and quercitin (Danilewicz *et al.*, 2008; Makhotkina & Kilmartin, 2009).

2.1.8 GLUTATHIONE

Glutathione (Fig 2.8) is a sulphur containing tripeptide (glycine-cysteine-glutamic acid) with a nucleophilic centre and is very important in the interactions of biological systems with their environment due to its detoxification actions. It protects organisms against toxicity and diseases connected to oxidative stress. This compound was first discovered in grapes in 1989 (Cheynier, 1989) and since then the role of glutathione in plants has been reviewed (Alscher, 1989). Glutathione already has established roles in limiting the effects of oxidation in juice and wine through its reaction with polyphenol *o*-quinones (Cheynier, 1993; Duboudieu *et al.*, 2000; Makhotkina & Kilmartin, 2009).

Glutathione is synthesized enzymatically in the grape berry by glutathione synthetase. During ripening, there is a dramatic increase in glutathione concentration during the rapid growth of the berries. In grape berries the reduced form of glutathione is the most abundant free thiol-containing compound at harvest.

Glutathione, with its electron-rich nucleophilic mercapto centre can spontaneously substitute by Micheal addition into the electrophilic centre of the *o*-quinone formed during oxidation. The product is a thioether, 2-S-glutathionyl-caftaric acid or Grape Reaction Product (GRP) (Fig 2.8). This leads to the regeneration of the vicinal dihydroxy ring of the phenol (Singleton *et al.,* 1985). The *o*-quinone reacting most readily with glutathione is that of caffeic acid (or *trans*-caftaric acid), while other phenols such as (+)-catechin or quercitin are also able to form glutathione derivatives. The cresolase activity of PPO converts coutaric acid to *trans*-caftaric acid and is then further oxidised and can thus result in GRP in the presence of glutathione (Singleton *et al.*, 1985).

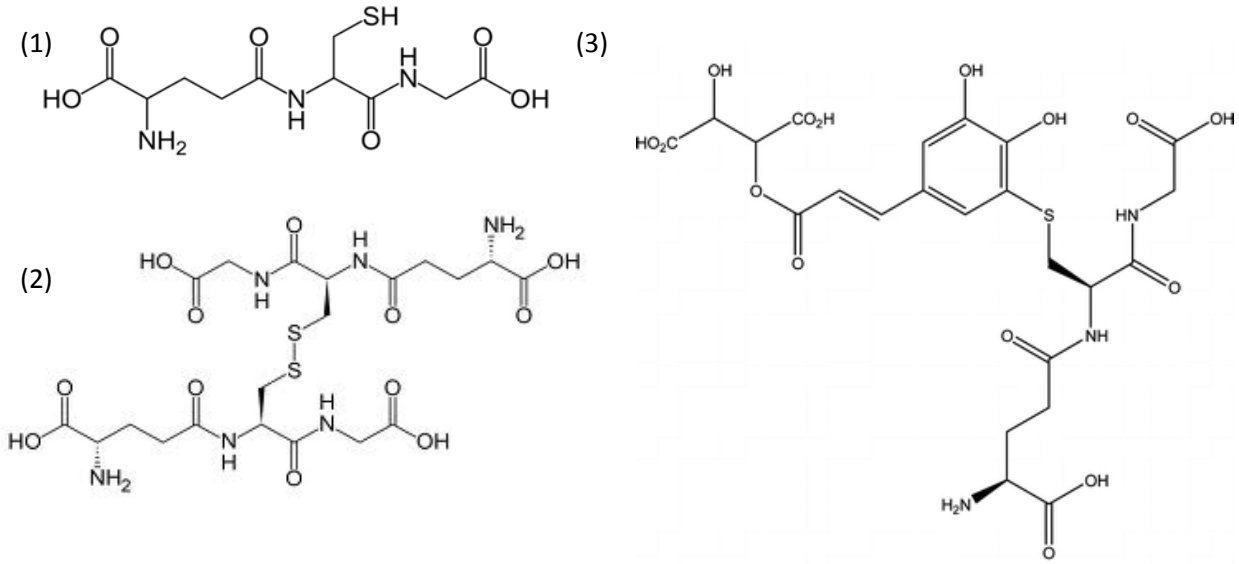


Figure 2.8 Reduced glutathione **(1)**, disulphide formed due to the oxidation of glutathione **(2)** and the grape reaction product **(3)**

The GRP is colourless and prevents browning of the must by trapping the *trans*-caftaric acid *o*-quinone. The addition of glutathione to the *o*-quinone is protective of white must by depleting the must of browning substrates, reducing the *o*-quinone back to the phenol and by substituting the *o*-quinone to regenerate the hydroquinone form of GRP (Bassil *et al.,* 2005).

The GRP is no longer a substrate for the PPO enzyme, but can be further oxidised by laccase. Laccase oxidises the GRP and yields the corresponding *o*-quinone which, in turn, can proceed to brown polymers and also give rise to 2,5-di-*S*-glutathionylcaftaric acid (GRP2) by a further addition of glutathione (Singleton *et al.*, 1985; Cheynier *et al.*, 1986; Salgeus *et al.*, 1986). As long as glutathione is available, GRP formation prevents the participation of *o*-quinones in coupled reactions leading to

pigment formation. GRP is susceptible to hydrolysis and can form hydrolysis products during aging (Cheynier *et al.*, 1986; Cejudo-Bastante *et al.*, 2010). Glutathione can in itself also be oxidised to form the disulphide (Fig 2.8) and this reaction could possibly be coupled with *o*-quinone reduction to a catechol group.

Other sulfhydryl compounds, such as cysteine and even hydrogen sulphide, can also act as antioxidants and react with *o*-quinones to form thio-ethers and regenerate colourless *o*-dihydroxyphenols (Singleton, 1987). However, these compounds' concentrations in must and wine is usually very low and its activity is negligible.

The reaction of the glutathione with *o*-quinone occurs in a 1.5:1 ratio. It appears that glutathione must be present at this or at a higher ratio at crushing to fully protect the must (Singleton *et al.*, 1985). The glutathione to caffeic acid ratio can serve as a good indication of the oxidation capacity of a medium. For berries and musts, this ratio can range from 1.3 to 12.7 and 0.6 to 10.5 respectively, with a lower ratio indicating a higher sensitivity towards browning. Due to glutathione's sensitivity to oxidation it has been proposed to be used as a marker of oxidation (Du Toit *et al.,* 2007).

Du Toit et al., 2007 tested the effect of reductive and oxidative treatments on the concentration of glutathione in white grape juice and found that the reductive treatments delivered the highest amounts glutathione. Control treatments that were exposed to about 1.0-1.5 mg/L of oxygen led to significantly lower contents of glutathione, even in the presence of 60 mg/L SO_2. Glutathione concentrations reduced drastically in oxidative treatments. In commercial wineries, oxygen consumption often exceeds this value, causing a large decrease in glutathione concentrations.

During crushing, the concentration of glutathione decreases rapidly because of the redox and enzymatic processes taking place (Adams & Liyanage, 1993). During pressing, the concentrations of glutathione was found to be high in the free run juices, but the concentration declined in the higher press fractions with an increase in the grape reaction product (GRP) (Maggu *et al.*, 2007; Patel *et al.*, 2010).

Glutathione concentrations usually decrease during fermentation. As very little oxidation can take place in this period, it is an indication that yeast plays a significant role in glutathione metabolism (Du Toit *et al.*, 2007; Patel *et al.*, 2010). Yeast strain, initial glutathione concentration and compositions of the must, are all factors that can influence the evolution of glutathione during fermentation.

2.1.8.1 EFFECT OF GLUTATHIONE ON AROMA COMPOUNDS

o-Quinones formed from oxidation of phenols, can bind with sulphur containing aroma compounds such as 3-sulfanylhexan-1-ol, 3-sulfanylhexyl acetate and 4-sulfanyl-4-methylpentan-2-one (which contribute to tropical and guava flavours in Sauvignon blanc) in the same manner as which glutathione associates with *o*-quinones. Glutathione could thus preserve these aroma compounds by competing with them for the addition to the *o*-quinone and so limiting the loss of varietal flavour. Wines produced from

oxygenated musts with glutathione addition, displayed significant quality improvement when compared to no glutathione addition (Vaimakis & Roussis, 1996).

The addition of glutathione and caffeic acid to a wine containing low amounts of sulphur dioxide, caused a decrease in the rate of disappearance of acetate esters, ethyl esters and terpenes during wine storage. The concentrations of total higher alcohols and fatty acids did not vary (Roussis *et al.*, 2007; Papadopoulou & Roussis, 2008). When this protected effect was tested in a model medium, it was found that glutathione inhibited the decrease of these volatile compounds in a dose-dependant manner. This effect was also observed with the addition of a mixture of N-acetyl-cysteine and caffeic acid (Roussis *et al.*, 2005; Sergianitis & Roussis, 2007). It therefore seems that the addition of glutathione to white wines before bottling could enhance the stability of the wine by preventing atypical wine aging.

Glutathione has been identified as a component of aromatic thiol precursors in grape must. The occurrence of glutathione could enlarge the precursor pool for potential positive odours (Peyrot Des Ganchos *et al.*, 2002a; Fedrizzi *et al.*, 2009; Capone *et al.*, 2010; Roland *et al.*, 2010b).

2.2 AROMA COMPOUNDS IN SAUVIGNON BLANC WINES

2.2.1 INTRODUCTION

Typical aroma of Sauvignon blanc can be described as vegetative, grassy, herbaceous, gooseberry, asparagus, green pepper, capsicum, tomato leaf, grapefruit and passion fruit (Swiegers *et al.,* 2006). Usually a Sauvignon blanc wine can be divided into two classes, “green” (vegetative, grassy, herbaceous, asparagus, green pepper, capsicum, tomato leaf) and “tropical” (gooseberry, grapefruit and passion fruit). The characteristics of this variety develop considerably during fermentation leading to very aromatic wines. Sauvignon blanc wines tends to be crisp and acidic on the palate and the winemaking technique usually excludes the use of oak.

The aroma of varietal wines, in this case Sauvignon blanc, arises from specific combination of odour-active aroma compounds. Only a few impact compounds are present in the juice in their free form. Methoxypyrazines and some of the monoterpenes can be found in the grapes, while compounds such as volatile thiols, some monoterpenes, esters, alcohols and fatty acids are either released from their precursors by yeast activity or occur as a result from yeast metabolism (Fischer, 2007).

2.2.2 METHOXYPYRAZINES

The very potent alkyl methoxypyrazines are the main compounds thought to be responsible for the “green” flavours found in Sauvignon blanc must and wine. Methoxypyrazines are nitrogen-containing ring structures (Fig 2.9) (Allen *et al.,* 1991) and result as a secondary product of amino acid catabolism in

the grape. The compound, 2-methoxy-3-isobutylpyrazine (ibMP) is considered to be the main contributor to the vegetative, grassy, green pepper, capsicum and asparagus-like aroma (Marais, 1994). Quantitatively ibMP has been found to be the pyrazine present in the highest concentrations in Sauvignon blanc musts and wines. Other pyrazines present at lower concentrations in Sauvignon blanc must and wine are 2-methoxy-3-isopropylpyrazine (ipMP) and to a lesser extend, 2-methoxy-3-sec-butylpyrazine (sbMP) (Lacey *et al.,* 1991). These compounds contribute to the asparagus and earthy aroma. All three compounds have a very low perception threshold of around 2ng/L in wine (Marais, 1994).

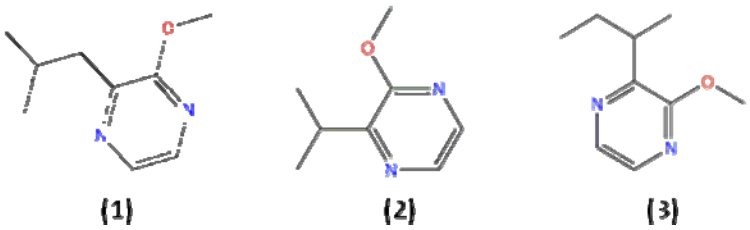


Figure 2.9 2-methoxy-3-isobutylpyrazine **(1)**, 2-methoxy-3-isopropylpyrazine **(2)** and 2-methoxy-3-sec-butylpyrazine **(3)** (Allen et al., 1991).

Pyrazine concentration will decrease during ripening, with maximum concentrations found at veriason (Lacey *et al.*, 1991). The concentration of these compounds at harvest is dependant on the origin and climate of the grapes. High temperatures promote the degradation of methoxypyrazines during ripening, therefore the concentration is lower in hot areas, while cooler climates such as the grape growing regions of New Zealand can deliver higher concentrations (Lacey *et al.,* 1988). Methoxypyrazines in maturing grapes are also light sensitive, subsequently shaded bunches can contain more of these compounds that sun-exposed bunches. Canopy management can be used to manipulate the amount of methoxypyrazines at harvest (Marais *et al.,* 1999).

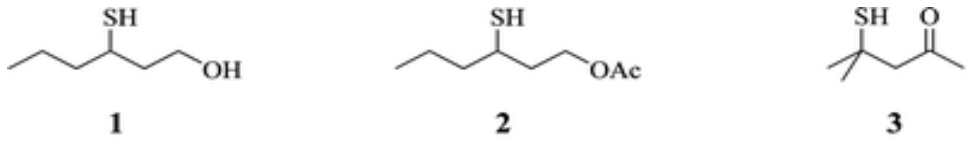
At harvest time, these compounds are mostly (95%) found in the skin of the grapes (Roujou De Boubée *et al.,* 2002), but is highly soluble in the juice aqueous solution. It has been found that the free run juice and the juice obtained in the early part of the pressing cycle, had the highest concentration of ibMP. During skin contact and during light pressing, the levels of ibMP increased slightly, but significantly, with no further increases at higher pressures (Marais, 1998; Roujou De Boubée *et al.*, 2002; Maggu *et al.*, 2007). It thus seems as if skin contact time has a greater influence than the amount of pressure applied (Maggu *et al.*, 2007). Winemaking procedures such as clarification of the must can cause a decrease in ibMP concentrations (Roujou De Boubée *et al.*, 2002).

Methoxypyrazines have been shown not to be very sensitive towards oxidative treatments. The addition of H_2O_2 to a neutral Chenin blanc wine, spiked with ibMP, did not lead to degradation of this compound over a three month period (Marais, 1998). Even hyperoxidation did not influence ibMP levels in Sauvignon blanc wines. It therefore appears that ibMP is resistant to oxidation (Marais, 1998).

2.2.3 THIOL CONTAINING COMPOUNDS

The volatile sulphur compounds in wine can be divided into two categories. On the one hand, certain volatile sulphur compounds may impart negative aromas such as a rotten egg originating from the formation of H_2S by wine yeast mostly from an inorganic sulphur source (Henschke & Jiranek, 1993; Rauhut, 1993). The production of secondary reductive odours can also contribute to negative off-odours such as cooked vegetables, onion and cabbage caused by sulphur containing compounds such as thioacetic acid esters and mercaptans and can happen due to too low redox potential of the wine (Rauhut, 1993; Brajkovich *et al.*, 2005).

However, other sulphur containing compounds can contribute to positive fragrances such as tropical, passion fruit and guava-like nuances (Goniak & Noble, 1987; Rauhut, 1993). These compounds were considered to be an impact odorant in Sauvignon blanc wines for the first time in 1993 and will hence be referred to as volatile thiols. These compounds are thiol containing compounds with additional functional groups such as ketones, alcohols and esters. Some volatile thiols (Fig 2.10) responsible for the fruity or tropical organoleptic flavours are 4-sulfanyl-4-methylpentan-2-one (4SMP) (Darriet *et al.,* 1995), reminiscent of box tree, passion fruit, broom and black current; and 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) (Tominaga *et al.,* 1996; Tominaga *et al.,* 1998a), responsible for the passion fruit, grapefruit and citrus aroma found in Sauvignon blanc wines. 4-Sulfanyl-4-methylpentan-2-ol (4SMPOH) can also contribute to the characters of citrus, passion fruit and grapefruit, although its organoleptic role is more limited, due to its concentration in wines seldom exceeding its olfactory threshold of 55 ng/L.



1 2 3

Figure 2.10 Structures of 3-sulfanylhexan-1-ol **(1)**, 3-sulfanylhexyl acetate **(2)**, 4-sulfanyl-4-methylpentan-2-one **(3)**

Perception thresholds for 4SMP, 3SH and SHA in model wine are 0.8 ng/L, 60 ng/L and 4.2 ng/L respectively (Tominaga *et al.,* 1998; Dubourdieu *et al.,* 2006) and have been reported in Sauvignon blanc wines from France and New Zealand in the range of 4-40 ng/L, 200-18000 ng/L and 0-2500 ng/L respectively (Ribéreau-Gayon *et al.*, 2006a; Lund *et al.*, 2009). The absolute and relative concentrations of these compounds in a wine will determine if the aroma perceived will be attractive or repulsive as these compounds often emit a sweaty or cat urine character when present at high concentrations (Swiegers *et al.*, 2006).

These compounds are not unique to Sauvignon blanc wines and have been found to contribute significantly to the aroma profiles of wines made from other varieties such as Riesling, Colombard, Semillon, Cabernet Sauvignon and Merlot (Tominaga *et al.,* 2000; Murat *et al.,* 2001a).

3SH is found in wines in two enantiomeric forms, the *R* form and *S* form (normally in a 50:50 ratio in dry white wines). These two enantiomers have almost the same perception threshold (50 and 60 ng/L for the *R* and the *S* form respectively), but differ slightly in the aroma that they present. The *R* form is reminiscent of grapefruit and citrus peel, while the *S* form contributes a passion fruit aroma. 3SHA also occurs in wine in two enantiomeric forms, but the *S* form (herbaceous, boxwood) has a perception threshold four times lower than the *R* form (passion fruit) and is normally found in concentrations three times more abundant (Tominaga *et al.,* 2006).

Unlike aroma compounds such as methoxypyrazines, volatile thiols are almost nonexistent in grape juice and are generated during the fermentation process by yeast from odourless, non-volatile precursors initially present in must (Darriet *et al.*, 1995; Tominaga *et al.*, 1996). 4SMP and 3SH are released during fermentation from precursors of which the cysteinylated [S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) and S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4SMP)] and glutathionylated [S-3-(hexan-1-ol)-glutathione (Glut-3SH) and S-4-(4-methylpentan-2-one)-glutathione (Glut-4SMP)] precursors have been identified, although these precursors only account for a fraction of the total amount of thiols found in the wine (Darriet *et al.*, 1995; Tominaga *et al.*, 1998b; Peyrot Des Ganchos *et al.*, 2002a; Subileau *et al.*, 2008; Fedrizzi *et al.*, 2009).

It is thought that the Glut-3SH arises from the conjugation of grape components glutathione and *(E)*-2-hexenal (Capone *et al.*, 2010). Glut-3SH is considered to be a “pro-precursor” as it is catalyzed to form Cys-3SH (Peyrot Des Ganchos *et al.*, 2002a). A proposed method of the formation of S-cysteine conjugate from the S-glutathione conjugate has been proposed (Peyrot Des Ganchos *et al.*, 2002a). S-glutathione conjugates are involved in the detoxification mechanisms of many living organisms. The toxic compound to be eliminated, conjugates with the glutathione molecule by S-glutathione transferase. The conjugated product is then broken down by removing glutamic acid by γ-glutamyltranspeptidase and glycine by carboxypeptidase, although these enzymes have never been identified in grapes (Subileau *et al.*, 2008). The formed product is the S-cysteine conjugate (Cys-3SH) (Wolf *et al.*, 1996; Peyrot Des Ganchos *et al.*, 2002a). The possibility of glutathione acting as an activator of 3MH release has also been raised (Subileau *et al.*, 2008). Work done by Patel *et al*., 2010 found an opposite trend as the addition of glutathione to grape must decreased the amount of 3SH and 3SHA formed during fermentation. It was concluded that the presence of glutathione could possibly repress thiol production by the yeast. This repression could be interpreted as competition by glutathione for the uptake of the thiol precursors by yeast (Patel *et al.*, 2010).

The precursors of 4SMP and 4SMPOH are mostly found in the juice at maturity, while the 3SH precursors are equally distributed between the juice and skins (Peyrot Des Ganchos *et al.*, 2002b). Skin

contact could thus lead to significant increases in the extraction of Cys-3SH, but has little effect on the extraction of Cys-4SMP and Cys-4SMPOH. The diffusion of the precursor into the liquid fraction of the must is slow, therefore a long maceration period is suggested (18 hours) if high 3SH precursors concentrations is sought after (Murat *et al.*, 2001b; Peyrot Des Ganchos *et al.*, 2002b; Maggu *et al.*, 2007). Higher temperatures during skin contact and high pressures during pressing, can further lead to increased concentrations of the 3SH precursor. The concentration of Cys-3SH increased 6.2 times in the juice obtained at 2 atmospheres when compared to the free run juice (Maggu *et al.*, 2007). Recent studies have also shown higher 3SH precursors in the higher pressed juices, but due to the higher oxidative potential of these juices, lower amounts of 3SH and 3SHA was found in the corresponding wines when compared to the wine made from free-run juices (Patel *et al.*, 2010).

In the juice, the concentration of Glut-3SH has been found to be up to 35 times higher than that of Cys-3SH and it thus seems that the cysteine conjugate is not the main precursor and 3SH probably mainly arose from glutathione conjugates (Capone *et al.*, 2010). Studies done by Subileau *et al.,* 2008 also supports the important role Glut-3SH has in volatile thiol formation. Grant-Preece *et al*., 2010 proved that Glut-3SH liberates 3SH forming Cys-3SH as an intermediate. This was done in a model fermentation and the same tendency is expected in grape must fermentations. Roland *et al*., 2010b, has also shown that Glut-3SH and Cys-3SH concentrations increased considerably from 7 days before the commercial harvest date to 7 days after. Glut-4SMP concentrations increased only slightly in this period, when compared to the concentrations of Glut-3SH and Cys-3SH. Like glutathione, the amount of volatile thiol precursors formed in the vine during ripening is enhanced by moderate water stress and non-limiting nitrogen levels (Peyrot Des Ganchos *et al.,* 2005).

During fermentation, the aromatic thiols are released from their non-aromatic precursors by yeast activity (Tominaga *et al.*, 1998b). Cysteine (Tominaga *et al.*, 1998b) and glutathione (Grant-Preece *et al.*, 2010; Roland *et al.*, 2010a) conjugates can generate odorous volatile thiols by the cleaving of the carbon-sulphur linkage (Murat *et al.*, 2001b; Dubourdieu *et al.*, 2006). This cleaving is thought to be caused by the C-S lyase enzyme as the level of 4SMP was reduced when the gene encoding for this enzyme was deleted from the yeast cell's genome. The probable role of multiple genes in the process of cleaving has also been investigated as no single key enzyme could be identified (Tominaga *et al.*, 1998a; Howell *et al.*, 2005). However, the exact mechanisms of transformation of cysteinylated and glutathionylated precursors by yeast into aroma compounds remain unknown. 3SHA is formed by the esterification of 3SH with acetic acid. This reaction is governed by yeast ester-forming alcohol acetyltransferase, which is encoded by the ATF1 genome (Swiegers *et al.,* 2007). Yeast strains differ in their ability to convert 3SH into 3SHA (Swiegers *et al.,* 2005).

There seems to be a limit in the ability of yeast to release the volatile thiols from the precursors. Calculations of the molar transformation yields from the cysteinylated precursor into the corresponding thiol yielded very low results and were variable. Only a small percentage of the precursors seem to be

ultimately transformed to the corresponding volatile thiol during fermentation. The conversion yield of Cys-3SH into 3SH and 3SHA has been found to be between 0.1 and 12%. An average level of transformation was found to be 1.4%, 3% and 4.2% for Cys-4SMP, Cys-4SMPOH and Cys-3SH respectively (Peyrot Des Ganchos *et al.,* 2000). Even with the consideration of the glutathionylated precursor as a precursor for the cysteinylated precursor, the yield would remain low (Subileau *et al.*, 2008). This indicates a possibility of other existing precursors not yet identified. There is therefore a large amount of aromatic potential locked up in the wine after fermentation, which can still be released in future with innovative winemaking techniques (Murat *et al.*, 2001b; Dubourdieu *et al.*, 2006; Masneuf-Pomarède *et al.*, 2006).

Yeast strains differ in their ability to release the volatile thiols from the odourless precursors. Research done at the Australian Wine Research Institute showed that the yeast strain VIN7 (Anchor Yeast) had the most potential of the tested commercial yeast strains as it produced the highest concentration of 4SMP and 3SHA, while VIN13 (Anchor Yeast) produced the highest concentration of 3SH. The '3SH-to-3SHA conversion capacity' of the different yeast strains was calculated by dividing the concentration of 3SHA by the concentration of 3SH. The yeast strain, QA23 (Lallemand) had the highest conversion capacity followed by NT116 (Anchor Yeast) and VL3 (Laffort Oenologie) (Swiegers *et al.*, 2006). The effect of different yeast strains on the production of 3SH in rosé wines made from Merlot, Cabernet franc and Cabernet Sauvignon has also been reported (Murat *et al.*, 2001b).

Fermentation temperature can also have an effect on the amount of thiols released from their precursors. In model ferments and in grape juice, it was found that the concentrations of 4SMP, 3SH and 3SHA were higher when fermentations were conducted between 18 ^{o}C and 20 ^{o}C when compared to fermentations conducted at 13 ^{o}C. Higher fermentation temperatures (23 ^{o}C and 28 ^{o}C) led to an initial increase in thiol concentrations, but decreased towards the end of fermentation (Masneuf-Pomarède *et al.*, 2006; Swiegers *et al.*, 2006).

3SH and 3SHA degrade during bottle ageing, particularly, 3SHA, which not only degrades, but also hydrolyses to form 3SH (Tominaga *et al.,* 2004). 3SHA was also identified as the least stable thiol compound in bottled wine monitored over time (Herbst *et al.,* 2008). Therefore, the role of 3SHA is more significant in young wines because of its fast degradation during aging. With the decline of antioxidants, free SO_2 and glutathione, a decrease in volatile thiols was also observed. There was also an increase in the free hydroxycinnamic acids, caffeic acid and p-coumaric acid due to hydrolysis from their tartaric acid esters. The flavanols, (+)-catechin and (-)-epicatechin had largely disappeared over time, corresponding to a loss in volatile thiols (Herbst *et al.*, 2008).

2.2.3.1 OXIDATION OF THE VOLATILE THIOLS AND THEIR PRECURSORS

Cysteinylated and glutathionylated precursors have thioether bonds which are quite stable. Because of this C-S bond the precursors are not sensitive to oxidation in the pre-fermentative stage of winemaking (Roland *et al.*, 2010b). In theory, the concentration of the precursors in the must should stay intact during oxidative handling. Interestingly, Roland *et al.,* 2010b reported a significant increase (140%) in the initial concentration of Glut-3SH after the addition of 2.1 mg/L oxygen to the juice. The production of Glut-3SH could be due to the addition of reduced glutathione on *(E)*-2-hexenal. *(E)*-2-Hexenal is a compound often unwanted in grape juice and wine, because of its connection to oxidation compounds and off-flavours and can be released from unsaturated fatty acids in the presence of lipoxygenase enzyme. It would seem as if it could possibly have a positive contribution to wine aroma due to its involvement in Glut-3SH production if present in low, but sufficient concentrations (Roland *et al.*, 2010b).

The same increase in Glut-4SMP would possibly be observed if mesityl oxide was present in pre-fermentative conditions. This compound has never been identified in grape juice, although its hydrate has been reported in wine at a mean level of 50 µg/L and in some Japanese grape varieties (Anon, 1996; Roland *et al.*, 2010b). When *(E)*-2-hexenal and/or glutathione were added to Sauvignon blanc juice with the addition of oxygen, an increase in Glut-3SH was observed. After fermentation, analyses showed a corresponding increase in of 3SH and 3SHA concentrations. Other studies have shown that the supplementation of glutathione to juice prior to fermentation, without the addition of oxygen, led to lower 3SH and 3SHA concentrations (Patel *et al.*, 2010). These studies concluded that pre-fermentative operations that were often considered as negative, such as juice exposure to oxygen, can have a positive contribution to the aromatic profile of the wine by increasing the amount of Glut-3SH present. However, the effects SO_2 additions in grape must have on the evolution of these compounds have not been investigated in detail.

Volatile thiols are highly reactive aroma compounds and studies have shown that their concentrations can possibly decrease by three mechanisms. The volatile thiols can oxidise easily in the presence of oxygen and iron to form its disulfide (Jocelyn, 1972; Kotserides *et al.*, 2000). Furthermore, these thiols are nucleophilic and capable of addition reactions with electrophiles such as polymeric phenolic compounds (like *trans*-caftaric acid) (Ribéreau-Gayon *et al.,* 1998, 2004b). This substitution reaction is especially reactive at low sulphur dioxide conditions (Blanchard *et al.*, 2004; Brajkovich *et al.*, 2005; Lopes *et al.*, 2009). These compounds can also participate in chemical reactions (Michael addition) with the products of phenolic oxidation (such as the *o*-quinones), in the presence of catalysts, iron and copper (Danilewicz, 2007; Herbst *et al.*, 2008; Nikolantonaki *et al.*, 2010).

During the oxidation of phenols, more specifically, catechols, *o*-quinones and semiquinone radicals are formed with the simultaneous formation of hydrogen peroxide from the reduction of oxygen, which

can be seen in Fig 2.11 (Danilewicz, 2003; Waterhouse & Laurie, 2006). The *o*-quinones formed are electrophilic and react with the nucleophilic thiol by a Micheal addition reaction (Fig 2.11 reaction 8) (Cheynier *et al.*, 1986). Singleton *et al*., 1985 tested the reactivity of a range of thiols and found that nearly all the compounds reacted in this manner. Oxidation reaction with the thiols can also occur via the generation of peroxides (Fig 2.11 reaction 9) (Wilderandt & Singleton, 1974).

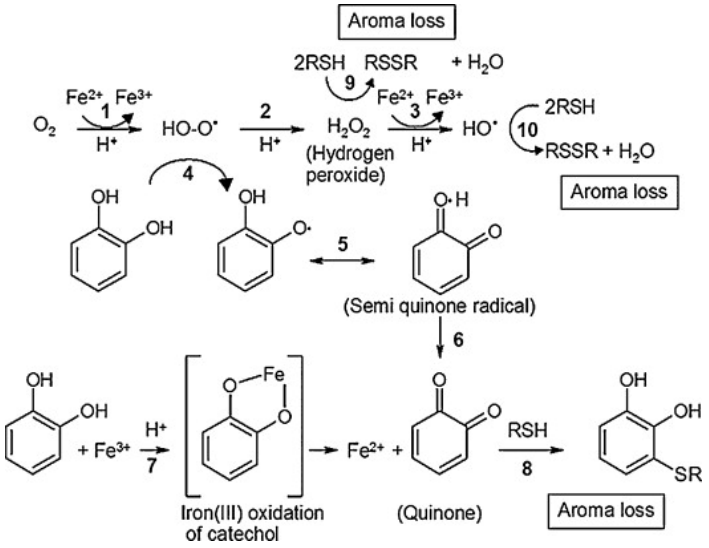


Figure 2.11 Possible mechanism for the oxidation of thiols (Nikolantonaki *et al.*, 2010)

3SH is both highly oxidizable and extremely reactive with *o*-quinones produced by the oxidation of phenolic compounds. The role of the polyphenol is undeniable as Blanchard *et al*., 2004 found a rapid decrease in 3SH concentration when in the presence of oxygen and (+)-catechin, than when only 3SH and oxygen was present. The *o*-quinone reacts with the thiol, rendering it odourless and causing a loss in varietal aroma. Nikolantonaki *et al.,* 2010 found the same tendency in model wine solutions, but with the addition of SO_2 the decrease was lower. In the study, they investigated the mechanism of oxidation of the thiols and compared the two possible mechanisms, which are the autooxidation of the thiol (Fig 2.11 reaction 9 and 10) and the addition reaction with the *o*-quinone (Fig 2.11 reaction 8). Experiments have shown that 3SH disappearance in oxygenated wine did not coincide with oxygen consumption, but only occurred 48 hours later (Darriet, 2002). Furthermore, disulphide formation was not observed in the more recent study (Nikolantonaki *et al.*, 2010). This confirmed the addition reaction involving the thiol and *o*-quinone. The addition of the thiol with the *o*-quinone was tested with two phenols. (+)-Catechin proved to be effective in being oxidised and react with the thiol. (-)-Epicatechin is a good oxygen consumer even in the presence of trace amounts of oxygen and reacts with oxygen more rapidly when compared to SO_2. Sulphur dioxide was unable to block the addition reaction between oxidised (-)-epicatechin and 3SH when compared to (+)-catechin where the protective effect of SO_2 was efficient in

blocking the reaction. It thus seems that SO_2 loses its antioxidant ability in the presence of (-)-epicatechin. Consequently, (-)-epicatechin is more reactive with 3SH than (+)-catechin.

The reactivity of the *o*-quinones with 4SMP was also tested. Results showed that the 4SMP molecule was rather stable against (+)-catechin. There were no drastic decreases with the presence of this phenol and oxygen. The concentration of 4SMP seemed to have decreased slightly over time, but this could have been caused by natural degradation. The protective effect of SO_2, was significant over time and inhibited this natural decrease when compared to wines where no SO_2 was present. In the presence of (-)-epicatechin and 4SMP, results were similar as with 3SH with a decrease in thiol concentration indicating higher reactivity of the *o*-quinone. Even though both thiols decreased in concentration, the decrease of 4SMP was lower than with 3SH, indicating less reactivity of the 4SMP molecule. It thus seems that 3SH reacts more readily with the formed *o*-quinones than 4SMP. This may be due to the structural properties of the thiols as 3SH is a secondary thiol and 4SMP tertiary. In this case, the position of the thiol and could have an effect due to steric hindrance (Kotserides *et al.*, 2000).

2.2.4 ACETATE AND ETHYL ESTERS

Other yeast derived aroma compounds can also have a significant effect on the aroma bouquet of a wine. Yeast can produce sensorial important volatile metabolites such as esters which mostly contribute to a pleasant smell in wine. The mixture of fermentation derived esters is mostly responsible for the fresh, fruity and even tropical aroma of young wines (Swiegers *et al.*, 2006). Of this group, the acetate esters largely contribute to the fruity, ester-like character of these wines (Marais & Pool, 1980). The ethyl esters of fatty acids can contribute to the complexity of a wine at low concentrations, but is often unwanted in higher concentrations due to odours of wax and honey. Very few esters are formed in grapes. They are mainly produced during fermentation by the condensation of an alcohol and a coenzyme-A-activated acid (acyl-CoA), by the action of alcohol acetyl transferases (Lambrechts & Pretorius, 2000). In a similar manner, ethyl esters are generated from acyl-CoA and ethanol. The alcohol reacts with an acid with the elimination of a water molecule during chemical estrification. A large variety of esters can be formed as all of the alcohols, especially ethanol, and fatty acids may react to form esters, leading to a large number of possible combinations. Acetate esters of the higher alcohols and the ethyl esters of straight-chain, saturated fatty acids are the most significant esters produced (Lambrechts & Pretorius, 2000). Some of the most qualitative significant esters have been identified to be isoamyl acetate, ethyl hexanoate and 2-phenylethyl acetate (Thurston *et al.,* 1981).

The production of volatiles (esters, higher alcohols and fatty acids) are not cultivar specific. The amount of volatiles present in Sauvignon blanc and Chardonnay wines were not significantly different except for hexyl acetate, hexanoic, decanoic and octanoic acid occurring in higher concentrations in Sauvignon blanc wines. This suggests that the volatile composition does not play a very important role in

the differentiation between these two wines and most probably other white varieties as well (Louw *et al.*, 2009).

There are many factors that can influence the formation of esters during fermentation. Fermentation conditions like temperature, juice clarification and yeast strain is but a few major impacting factors. The reason for contradicting results in literature could possibly be the different reactions of individual yeast strains to the fermentation conditions (Bertrand, 1968).

During grape processing, aeration often occurs as a result of its transfers in industrial processes. Yeast metabolism is modified by the presence of oxygen, enhancing the fermentation by increasing sterol biosynthesis and favouring cellular growth (Larue *et al.,* 1980). This enables the yeast cells to improve ethanol tolerance, fermentative capability and viability (Valero *et al.,* 2001). Not only is the yeast growth enhanced, but is seems as if there could be a close relationship between the fermentation rate and ester production (Nordström, 1965; Houtman *et al.*, 1980).

In literature, results are often contradicting when investigating the effect of different winemaking techniques on the formation of volatile compounds such as esters, higher alcohols and fatty acids. An increase in major esters were found in wines made from must with prior oxygenation (Valero *et al.*, 2002). This is thought to be due to the increased growth rate of the yeast. Another study found an increased production of isoamyl actetate and phenethyl acetate in semi-aerobic fermentation conditions when compared to anaerobic conditions. However, the application of aerobic conditions was not aggressive and it only allowed the fermenting juice to be in contact with air. The addition of oxygen to the must have also been found to increase ethyl ester concentrations, but decreased the concentrations of some acetate esters (Bertrand & Torres-Alegre, 1984). In contrast, an increase of isopentyl acetate, ethyl hexanoate, ethyl octanoate and phenethyl acetate was observed in anaerobic conditions when compared to semi-aerobic conditions (Nykänen, 1986).

When determining the concentration of volatile aroma compounds of wines made with different SO_2 additions, a variation in results were also found. Marais, 1998 and Garde-Cerdán *et al*., 2007 could not find consistent differences between the ester content in wines made from juice with and without SO_2 addition prior to fermentation. However, in other studies, higher acetate and ethyl ester concentrations was found in wines made with SO_2 (Daudt & Ough, 1973; Moio *et al.*, 2004) and hyperoxidised juice (Van Wyk *et al.,* 1996). This could possibly be due to the difference in sensitivity to SO_2 of specific enzymes responsible for the formation of the volatile compounds (Daudt & Ough, 1973).

The effect of wine storage or oxygen addition to wine, on volatile aroma compounds have been investigated extensively as it is known to cause a general decrease in positive aroma compounds with an increase in unwanted flavours (Simpson, 1978; Marais *et al.*, 1992; Boulton *et al.*, 1996; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003). Volatile esters are very unstable, due to the equilibrium in a wine medium favouring hydrolysis. However, the precise mechanism that causes esters to disappear during wine storage is still unclear. Oxidation processes or other chemical reactions might lead to losses in

volatile compounds. Proposed explanations are that ester concentrations change due to hydrolysis and estrification (Ramey & Ough, 1980) or oxidation by hydroxyl-radical oxidation related processes (Litchev, 1989; Escudero *et al.*, 2000). There are no apparent method, other than low storage temperatures, that can help stabilize these esters. Low fermentation temperatures of about 15 ^{O}C encourage the formation of volatile esters by the yeast (Killian & Ough, 1979). It seems that clarified juice is also advantageous for the formation of esters as lower concentrations of the hydrolyses enzyme, esterase, occurs.

The initial post fermentation concentrations of esters differ from wine to wine. Individual ester behaviour can thus vary for each wine due to the ester hydrolysis-estrification equilibrium which is dependant on the original concentrations of esters present. However, some generalizations can be made: acetate esters of higher alchohols tends to diminish during aging more rapidly than ethyl esters of fatty acids, and the ethyl esters of polyprotic acids such as tartaric and succinic, which are not appreciably synthesized by yeast, may be chemically estrified during the course of aging. The longer the chain of the fatty acid, the faster the hydrolysis rate becomes. Some esters were found to be relatively stable with a slight or no changes during aging. Higher SO_2 concentrations preserved the aroma composition better during storage (Ramey & Ough, 1980; Ough, 1985).

2.2.5 HIGHER ALCOHOLS

Higher alcohols (also known as fusel alcohols) are alcohols that have more than two carbon atoms in their structure with a higher molecular weight and boiling point than ethanol (Lambrechts & Pretorius, 2000). They are secondary products of alcoholic fermentation and can be important precursors for ester formation (Soles *et al.,* 1982). These alcohols and their esters have a large influence on the aromatic composition of wines as they often display intense and pleasant flavours when present at total concentrations higher than 300 mg/L. However, excessive concentrations (higher than 400 mg/L) can result in a strong, pungent smell and taste, whereas optimal levels impart fruity characters (Rapp & Mandery, 1986). With the exception of 2-phenyl ethanol, which has rose and floral aromas, higher alcohols in general often display unpleasant fusel and solvent-like aromas when present in high concentrations (Lambrechts & Pretorius, 2000).

Higher alcohols are formed by the yeast during alcoholic fermentation. It can be anabolically synthesized from intermediates of the sugar metabolism or catabolically synthesized from branch-chain amino acids, through the Ehrlich pathway (Nykänen, 1986; Boulton *et al.*, 1996; Dickinson *et al.*, 1997; Dickinson *et al.*, 2003). Amino acids can undergo a process of oxidation, hydrolyzation and decarboxylation to produce these higher alcohols.

Butanol and pentanol are formed from a keto acid that takes part as an intermediate in cell glucidic metabolism. Fermentative conditions that cause diminished yeast cell growth would result in the production of these alcohols. This is probably due to its biosynthetic pathway since carboxylations

would be favoured by the absence of oxygen (Mauricio *et al.*, 1997). Metabolic pathways involving amino acids are responsible for the production of isoamyl alcohol, isobutyl alcohol and 2-phenyl ethanol. The production of these alcohols depends on the cellular growth and the presence of oxygen in the medium (Mauricio *et al.*, 1997).

Hexanol and hexenol are six carbon alcohols that can originate from the grapes. These alcohols are responsible for the typical grassy, leafy and herbaceous-like character in unripe grapes (Marais, 1994). During crushing, the grape skins break open and juice comes in contact with oxygen. Lipoxygenase action catalyses the oxidation of unsaturated fatty acids (linoleic or linolenic acid) and produces *(E)*-2-hexenal and derivatives, contributing to herbaceous and grassy odours (Boulton *et al.*, 1996). This effect often occurs when the grapes are harvested at insufficient grape maturity (Ribéreau-Gayon *et al.*, 2006a). *(E)*-2-Hexenal is generated in the must from a few to hundreds of micrograms per litre, depending on grape variety and pre-fermentative treatments (Cordonnier & Bayonove, 1981). The yeast reduces hexyl aldehyde to hexanol and can then convert it to hexyl acetate (Boulton *et al.*, 1996). This could lead to higher concentrations of hexanol and hexanal being formed from linoleic acid (Drawert *et al.,* 1966). These six carbon alcohols are depleted within the first few days of alcoholic fermentation (Mauricio *et al.*, 1997).

Fermentation conditions and the amount and type of amino acids present and the yeast strain will determine the amount and variety of alcohols formed. In general, conditions that increase the fermentation rate, such as oxygenation and higher temperatures will also increase the formation of higher alcohols. Valero *et al*., 2002 investigated the effect of oxygen addition prior to fermentation on higher alcohol production and found a increase in higher alcohols in wines made from must with prior oxygenation (Zoecklein *et al.*, 1995; Valero *et al.*, 2002). This was not the case for all of the alcohols as the level of hexanol was similar for oxygenated and non-oxygenated musts. The production of butanol and propanol was also greater with the absence of must oxygenation (Valero *et al.*, 2002). Production of ethanol, isoamyl alcohol, isobutyl alcohol and 2-phenyl ethanol was also increased in semi-aerobic fermentation conditions when compared to anaerobic conditions. The production of pentanol and butanol was delayed compared to anaerobic fermentation (Mauricio *et al.*, 1997). The general increase in higher alcohols can be ascribed to increased growth and biosynthetic activity of yeast in the presence of oxygen.

The formation of isoamyl alcohol and 2-phenyl ethanol was significantly greater in a fermentation conducted with SO_2 when compared to a fermentation conducted without this anti-oxidant (Garde-Cerdán & Ancín-Azpilicueta, 2007). The same explanation as for the esters is proposed (Daudt & Ough, 1973).

During aging of Chenin blanc wines, the concentrations of 2-phenyl ethanol and the amyl alcohols decreased significantly, possibly due to the oxidation of the alcohols to aldehydes. Increases were observed for isobutanol and hexanol (Marais & Pool, 1980). More recent studies reported stable higher

alcohol concentrations during the aging of Muscat and Debina wines (Roussis *et al.*, 2005; Roussis *et al.*, 2007).

2.2.6 FATTY ACIDS

Volatile acidity refers to a group of volatile organic acids of short carbon chain-length. The volatile fatty acid content of wine usually lies between 500 and 1000 mg/L of which about 90% is acetic acid (Hensche, 1993; Radler, 1993). Although yeast can also produce a small amount of acetic acid, elevated levels is usually associated with bacterial spoilage (Ribéreau-Gayon *et al.*, 2006b). Propionic acids and butyric acids are by-products of fermentation and are also usually associated with bacterial spoilage (Ribéreau-Gayon *et al.*, 2006b). Higher values of volatile acidity were found in wines made from oxygenated musts with no SO_2 additions. This is probably due to the growth of acetic acid bacteria in the presence of oxygen (Vaimakis & Roussis, 1996).

Low concentrations fatty acids can contribute positively to the complexity of wines. Fatty acids are essential constituents of the plasma membrane and are precursors of more complex molecules. They can be synthesized by the repetitive condensation of acetyl-CoA by the action of the fatty acid synthetase complex (Lambrechts & Pretorius, 2000). Medium chain fatty acids (MCFA) and their ethyl esters are natural components of wine and can play a key role in the fruity notes of a wine. MCFA such as hexanoic, octanoic and decanoic fatty acids are produced by the yeast as intermediates in the biosynthesis of long chain fatty acids. This takes place in the early stages of alcoholic fermentation especially with clarified musts and in the absence of oxygen (Houtman & Du Plessis, 1986). These compounds can inhibit fermentation at elevated concentrations and cause stuck or sluggish fermentations as they are toxic to yeast cells (Bardi *et al.,* 1999). Hexanoic, octanoic and decanoic acids accumulate in the yeast with anaerobic conditions and can be secreted in the wine (Bardi *et al.*, 1999). However, the presence or absence of SO_2 had no significant effect on the formation of MCFA (Garde-Cerdán & Ancín-Azpilicueta, 2007). During the aging of Muscat wines, no significant difference in MCFA concentrations was observed (Roussis *et al.*, 2005).

Long chain unsaturated fatty acids, such as oleic acid and linoleic acid can enhance fermentations, but are not yeast derived products and originate from the waxy cuticle of grape skins. They are essential precursors for the formation of lipid compounds found in yeast (Lambrechts & Pretorius, 2000).

2.2.7 MONOTERPENES

Monoterpenes and monoterpene alcohols are 10-carbon compounds, many of which are volatile and odorous. They are known for their floral, fruity, citrus and perfume odours usually expressed by geraniol, linalool, nerol and α-terpineol (Marais, 1983). One of the most common monoterpenes found in Sauvignon blanc grapes are α-terpineol.

Terpenes are not considered to be a character- impacting compound for Sauvignon blanc as they are usually expressed as an impact odorant for the muscat family of grapes (Ribéreau-Gayon *et al.*, 2006b). However, relatively high concentrations of free *trans*-pyran linalool oxide, *cis*-8-hydroxy-linalool, 2-hydroxy-1,8-cineole, diendiol-1, metenediol-2 and bound α-terpineol where found in Sauvignon blanc grapes when compared to Weisser Riesling (Versini *et al.,* 1992). Even though terpene concentrations in wine such as Sauvignon blanc, are generally below the perception threshold, the olfactory impact of terpene compounds is often synergistic and can have an impact on the overall complexity of a wine (Ribéreau-Gayon *et al.*, 2006b).

Terpene concentrations in general increase during ripening and start to decrease when the grapes reach an overripe stage (Marais, 1983). A considerable proportion of these compounds are in bound form in the juice, known as aglycons (or glycosides). The hydrolysis of the *O*-glycosyl bond during fermentation by means of yeast β-glycosidases, will release the terpenoid compound, rendering it aromatic, although some aglycons can become aromatic by chemical rearrangement (Williams *et al.,* 1980; Loscos *et al.,* 2007) Exogenous enzymes are often added to juice, due to natural enzymes not performing optimally at wine pH. Free monoterpenes are also present in the grape at varying concentrations, depending on the specific compound. In general, more bound glycosides are found than the free terpenoids, and the ratios of bound to free terpenoids can vary amongst different grape cultivars.

The bound glycosylated monoterpenes and the free monoterpenes are mostly located in the grape skin (Ribéreau-Gayon *et al.*, 2006b). Some important terpenes are located in the juice while others, such as α-terpineol are evenly distributed between the skin and juice (Marais, 1983). Extraction of monoterpenes from the skins are enhanced with longer skin contact times as well as high skin contact temperatures (Marais & Rapp, 1988; Marais, 1998).

Fermentation conditions that stimulate the glycolytic flux, such as high assimilable nitrogen content and aerobic fermentation conditions, often results in the higher concentrations of monoterpenes found in the wines. Terpenes that were mainly synthesized under these conditions were linalool, α-terpineol, citronellol and geraniol. α-Terpineol were produced in the highest concentrations and considered to be the main product of chemical and/or biological transformation of linalool (King & Dickinson, 2000). Biotransformation of linalool, α-terpenol, nerol, ho-trienol and geraniol by *S. cerevisiae* has also been reported (King & Dickinson, 2000). These results further suggest that a *de novo* synthesis of monoterpenes by *Saccharomyces* yeast strains takes place.

However, after 15 hours skin contact, settled juices with and without prior SO_2 additions did not show any significant differences in the total terpene content (Marais, 1998).

Oxidation of Muscat wines can cause a remarkable loss of aroma and higher terpene concentrations were found in wines that were made in a CO_2 enriched atmosphere (Versini *et al.,* 1981). Terpenes are sensitive to acidic conditions, storage time and temperature and can be transformed to

other compounds which could portray a different aroma. Most monoterpene alcohols are replaced by terpene oxides during oxidation. These oxides have a perception threshold of about 10 times higher than the corresponding alcohols (Papadopoulou & Roussis, 2001). During maturation terpenes can undergo transformation and the total terpene content can decrease. Linalool and α-terpineol were two compounds found to decrease significantly during storage (Roussis *et al.*, 2005; Roussis *et al.*, 2007).

2.3 CONCLUSIONS

It is clear that oxygen can play an important role during white wine production. Oxygen causes oxidation reactions to occur and produce products such as *o*-quinones and H_2O_2, which can induce polymerization reactions in juice and wines. These oxidation products can react with other juice and wine components, such as antioxidants and even aroma compounds. Sulphur dioxide plays an important role in protecting juice and wine from oxidation.

Fermentation by *Saccharomyces cerevisiae* also has a great impact on wine as it increases the chemical and aroma complexity by modifying some grape derived compounds and producing a substantial amount of yeast metabolites. The yeast potential to form these metabolites is greatly influenced by the fermentation conditions. The presence of oxygen and sulphur dioxide can have varying effects on the ability of the yeast to perform.

From grassy and herbaceous aromas to a citrus and tropical flavour mixture, Sauvignon blanc displays very unique organoleptic combinations. Of these compounds, the methoxypyrazines and the volatile thiols are considered to be the most character impacting compounds of this variety. For the winemaker, it becomes increasingly important to maintain the typical Sauvignon blanc aroma and optimise the winemaking techniques to preserve these flavours and the role of O_2 in this process must not be underestimated.

2.4 LITERATURE CITED

Adams, D. O. & Liyanage, C., 1993. Glutathione increases in grape berries at the onset of ripening. Am. J. Enol. Vitic. 44, 333-338.

Allen, M. S., Lacey, M. J., Harris, R. L. N. & Brown, W. V., 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. Am. J. Enol. Vitic. 42 (2), 109-112.

Alscher, R. G., 1989. Biosynthesis and antioxidant function of glutathione in plants. Physiol. Plant. 77, 457-464.

Amano, Y., Kubota, M. & Kagami, M., 1979. Oxygen uptake of Koshu grape must and its control. Hokkokogaku Kaishi. 57, 92-101.

Anon. 1996. TNO, Volatile compounds in foods. Qualitative and quantitative data. Nutrition and food research institute, Zeist, The Netherlands. 823.

Bardi, L., Cocito, C. & Marzona, M., 1999. *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeriation and in abscence of exogenous lipids. Int. J. Food Microbiol. 47, 133-140.

Bassil, D., Makris, D. & Kefalas, P., 2005. Oxidation of caffeic acid in the presence of L-cysteine: isolation of 2-S-cysteinylcaffeic acid and evaluation of its antioxidant properties. Food Res. Int. 38, 395-402.

Bertrand, A., 1968. Utilisation de la chromatographie en phase gazeuse pour le dosage des constituants volatils du vin. Thesis, University of Bordeaux.

Bertrand, A. & Torres-Alegre, V., 1984. Incidence de L'action de L'oxygène sur la formation des produits secondaires de la fermentation alcoolique du moût de raisin. Science des Aliments. 4, 45-64.

Betés-Saura, C., Andrés-Lacueva, C. & Lamuela-Raventós, R. M., 1996. Phenolics in white free run juices and wines from Penedès by high-performance liquid chromatography: changes during vinification. J. Agric. Food Chem. 44, 3040-3046.

Blanchard, L., Darriet, P. & Dubourdieu, D., 2004. Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. Am. J. Enol. Vitic. 55, 115-120.

Boulton, R. B., 2003. A radical view of oxidative reactions in wine. In: Pszczólkowski, P. (ed). Proc. Congreso latinoamericano de viticultura y enología. Pontificia Universidad Católica de Chile, Santiago. pp.

Boulton, R. B., Singleton, V. L., Bisson, L. F. & Kunkee, R. E., 1996. Principles and practice of winemaking. Chapman & Hall, New York.

Bradshaw, M. P., Scollary, G. R. & Prenzler, P. D., 2001. Ascorbic acid-induced browning of (+)-catechin in a model wine system. J. Agric. Food Chem. 49, 934-939.

Brajkovich, M., Tibbits, N., Peron, G., Lund, C. M., Dykes, S. I., Kilmartin, P. A. & Nicolau, L., 2005. Effect of screwcap and cork closures on SO_2 levels and aromas in Sauvignon blanc wine. J.Agric. Food Chem. 53 (26), 10006-10011.

Capone, D. L., Sefton, M. A., Hayasaka, Y. & Jeffery, D. W., 2010. Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS: Resolution and quantitation of diastereomers of 3-*S*-Cysteinylhexan-1-ol and 3-*S*-Glutathionylhexan-1-ol. J. Agric. Food Chem. 58, 1390-1395.

Cejudo-Bastante, M. J., Pérez-Coello, M. S. & Hermosín-Gutiérrez, I., 2010. Identification of new derivatives of 2-S-Glutathionylcaftaric acid in aged white wines by HPLC-DAD-ESI-MSn. J. Agric. Food Chem.,

Cheynier, V., Fulcrand, H.H., Guyot, S., Souquet, J.M., Moutounet, M., 1995. In: (ed). Proc. 4th International Symposium on Innovation in Enology. Messe, Stuttgart, Killesberg. pp. 50

Cheynier, V., Masson, G., Rigaud, J., Moutounet, M., 1993. Estimation of must oxidation during pressing in Champagne. Am. J. Enol. Vitic. 44 (4), 393-399.

Cheynier, V., Osse, C. & Rigaud, J., 1988. Oxidation of grape juice phenolic compounds in model solutions. J. Food Sci. 53, 1729-1732.

Cheynier, V., Rigaud, J., Souquet, J. M., Duprat, F. & Moutounet, M., 1990. Must browning in relation to the behaviour of phenolic compounds during oxidation. Am. J. Enol. Vitic. 41, 346-349.

Cheynier, V., Souquet, J.M., Moutounet, M., 1989. Glutathione content and glutathione to hydroxycinnamic acid racio in *vitis vinifera* grapes and musts. Am. J. Enol. Vitic. 40, 320-324.

Cheynier, V., Trousdale, E., Singleton, V. L., Salgeus, M. & Wylde, R., 1986. Characterization of 2-S-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. J. Agric. Food Chem. 34, 217-221.

Cilliers, J. J. L. & Singleton, V. L., 1989. Nonenzymatic autooxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37, 890-896.

Cordonnier, R. & Bayonove, C., 1981. Etude de la phase perfermentaire de la vinification: extraction et formation de certains composés de l'arôme; cas des terpenols, des aldehydes et des alcohols en C6. Connaiss. Vigne Vin. 15, 269-286.

Danilewicz, J. C., 2003. Review of Reaction Mechanisms of Oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper. Am. J. Enol. Vitic. 54, 73-85.

Danilewicz, J. C., 2007. Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. Am. J. Enol. Vitic. 58 (1), 53-60.

Danilewicz, J. C., Seccombe, J. T. & Whelan, J., 2008. Mechanism of interaction of polyphenols, Oxygen, and Sulphur dioxide in model wine and wine. Am. J. Enol. Vitic. 59 (2), 128– 136

Darriet, P., 2002. Caracterisation des composes volatils associes a la vigne et au vin. Applications technologiques. Universite Victor Segalen Bordeauxm. p 97.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J. & Dubourdieu, D., 1995. Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-Mercapto-4-methylpentan-2-one. Flavour and Fragrance Journal. 10, 385-392.

Daudt, C. E. & Ough, C. S., 1973. Variations in some volatile acetate esters formed during grape juice fermentation temperature, SO_2, Yeast strain, and grape variety. Am. J. Enol. Vitic. 24 (3), 130-135.

Dickinson, J. R., Lanterman, M. M., Danner, D. J., Paerson, B. M., Sanz, P., Harrison, S. J. & Hewlins, J. E., 1997. A 13C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. J. Biol. Chem. 272 (43), 26871-26878.

Dickinson, J. R., Salgado, L. E. & Hewlins, J. E., 2003. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. J. Biol. Chem. 278 (10), 8028-8034.

Drawert, F., Heimann, W., Emberger, R. & Tressl, R., 1966. Enzymatische Bildung von Hexen-(2)-al-(1). Liebg. Ann. Chem. 694.

Du Toit, W. J., 2003. Winemaking with rotten grapes: it can be a headache. Wynboer. 161, 81-83.

Du Toit, W. J., Lisjak, K., Stander, M. & Prevoo, D., 2007. Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. J. Agric. Food Chem. 55 (8), 2765-2769.

Du Toit, W. J., Marais, J., Pretorius, I. S. & Du Toit, M., 2006. Oxygen in wine: A review. S. Afr. J. Enol. Vitic. 27 (1), 76-94.

Dubernet, M. & Ribéreau-Gayon, P., 1973. Presence et significance dans les mouts et les vins de la tyrosinase du raisin. Conn. Vigne Vin. 7, 283-302.

Dubernet, M. & Ribéreau-Gayon, P., 1974. Causes et consequences de la consummation de l'oxygene par les mouts des raisin. Vitis. 13, 233-244.

Duboudieu, D., Moine-Ledoux, V., Lavigne-Cruege, V., Blanchard, L. & Tominaga, T., 2000. Recent advances in white wine aging: the key role of the lees. In: (ed). Proc. ASEV 50th Anniversary Meeting. Seattle, WA. pp.

Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot Des Gahons, C. & Murat, M. L., 2006. The role of yeast in grape flavour development during fermentation: The example of Sauvignon blanc. Am. J. Enol. Vitic. 57 (1), 81-88.

Escudero, A., Asencio, E., Cacho, J. & Ferreira, V., 2002. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chem. 77, 325-331.

Escudero, A., Hernandez-Orte, P., Cacho, J. & Ferreira, V., 2000. Clues about the role of methional as character impact odorant of some oxidized wines. J. Agric. Food Chem. 48, 4268-4272.

Fedrizzi, B., Pardon, K. H., Sefton, M. A., Elsey, G. M. & Jeffery, D. W., 2009. First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan-2-one, in Sauvignon blanc juice. J. Agric. Food Chem. 57 (3), 991-995.

Fenton, H. J. H., 1894. Oxidation of tartaric acid in presence of iron. J. Chem. Soc. 75, 1-11.

Fischer, U., 2007. Flavours and Fragrances : Chemistry, Bioprocessing and Sustainability. Springer Berlin Heidelberg, Berlin Heidelberg.

Garde-Cerdán, T. & Ancín-Azpilicueta, C., 2007. Effect of SO_2 on the formation and evolution of volatile compounds in wines. Food Control. 18 (12), 1501-1506.

Goniak, O. J. & Noble, A. C., 1987. Sensory study of selected volatile sulfur compounds in white wine. Am. J. Enol. Vitic. 38, 223-227.

Grant-Preece, P. A., Pardon, K. H., Capone, D. L., Cordente, A. G., Sefton, M. A., Jeffrey, D. W. & Elsey, G. M., 2010. Synthesis of wine thiol conjugates and labeled analogues: Fermentation of the glutathione conjugate of 3-mercaptohexan-1-ol yields the corresponding cysteine conjugate and free thiol. J. Agric. Food Chem. 58, 1383-1389.

Green, M. J. & Hill, H. A. O., 1984. Chemistry of dioxygen. Methods Enzymol. 105, 3-22.

Haisman, D. R., 1974. The effect of sulphur dioxide on oxidising enzyme systems in plant tissues. J. Sci. Food Agr. 25, 803.

Hensche, P. A., Jiranek, V., 1993. Yeasts: Growth during fermentation. In*:* Fleet, G. H. (eds). Wine Microbiology and biotechnology, Hardwood academic publishers, Chur, Switzerland. pp. 27-54.

Henschke, P. A. & Jiranek, V., 1993. Yeasts: Growth during fermentation. In*:* Fleet, G. H. (eds). Wine Microbiology and biotechnology, Hardwood academic publishers, Chur, Switzerland. pp. 27-54.

Herbst, M., Kilmartin, P. A. & Nicolau, L., 2008. Aroma stabilty in Sauvignon blanc wines. The Australian and New Zealand Grapegrower and Winemaker 6 (Annual Technical Issue), 66-72.

Hornsey, I., 2007. The chemistry and biology of winemaking The Royal Society of Chemistry, Cambridge, UK.

Houtman, A. C. & Du Plessis, C. S., 1986. The effect of grape cultivar and yeast strain on fermentation rate and concentration of volatile components in wine. S. Afr. J. Enol. Vitic. 7 (3), 14-20.

Houtman, A. C., Marais, J. & Du Plessis, C. S., 1980. The possibilities of applying present-day knowledge of wine aroma components: Influence of several juice factors on fermentation rate and ester production during fermentation. S. Afr. J. Enol. Vitic. 1 (1), 27-33.

Howell, K. S., Klein, M., Swiegers, J. H., Hayasaka, Y., Elsey, G. M., Fleet, G. H., Hoj, P. B., Pretorius, I. S. & De Barros Lopez, M. A., 2005. Genetic determinants of volatile thiol release by Saccharomyces cerevisiae during wine fermentation. Appl. Environ. Microbiol. 71, 5420-5426.

Jocelyn, P. C., 1972. Biochemistry of the SH group. The occurance, chemical properties, metabolism and biological function of thiols and disulphides. Academic Press, London, UK.

Killian, E. & Ough, C. S., 1979. Fermentation esters-Formation and retention as affected by fermentation temperature. Am. J. Enol. Vitic. 30, 310-305.

King, A. & Dickinson, J. R., 2000. Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis*. Yeast. 16, 499-506.

Kotserides, Y., Ray, J., Augier, C. & Baumes, R., 2000. Quantitative determination of sulfur containing wine odorants at sub-ppb levels. 1. Synthesis of the deuterated analogues. J. Agric. Food Chem. 48, 5819-5823.

Lacey, M. J., Allen, M. S., Harris, R. L. N. & Brown, W. V., 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. Am. J. Enol. Vitic. 42, 103-108.

Lacey, M. J., Brown, W. V., Allen, M. S. & Harris, R. L. N., 1988. Alkyl methoxypyrazines and Sauvignon blanc character. In: (ed). Proc. Cool Climate Vitic. Oenol. Symp., Auckland, New Zealand.

Lambrechts, M. G. & Pretorius, I. S., 2000. Yeast and its importance to wine aroma - A review. S. Afr. J. Enol. Vitic. 21 (Special Issue), 97-129.

Larue, F., Lafon-Lafourcade, S. & Ribéreau-Gayon, P., 1980. Relationship between the sterol content of yeast cells and their fermentation activity in grape must. Appl. Environ. Microbiol. 39, 808-811.

Litchev, V., 1989. Influence of oxidation processes on the development of the taste and flavor of wine distillates. Am. J. Enol. Vitic. 40, 31-35.

Lopes, P., Silva, M. A., Pons, A., Tominaga, T., Lavigne, V., Saucier, C., Darriet, P., Teissedre, P. L. & Dubourdieu, D., 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. J. Agric. Food Chem. 57, 10261-10270.

Loscos, N., Hernandez-Orte, P., Cacho, J. & Ferreira, V., 2007. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions J. Agric. Food Chem. 55, 6674-6684.

Louw, L., Roux, K., Tredoux, A., Tomic, O., Naes, T., Nieuwoudt, H. H. & Van Rensburg, P., 2009. Characterization of selected South African young cultivar wines using FTMIR Spectroscopy, Gas Chromatography, and Multivariate Data Analysis. J. Agric. Food Chem. 57, 2623-2632.

Lu Valle, J. E., 1952. The reaction of quinone and sulfite. I. Intermediates. J. Am. Chem. Soc. 72, 2970-2977.

Lund, C. M., Thompson, M. K., Benkwitz, F., Wohler, M. W., Triggs, C. M., Gardner, R., Heymann, H. & Nicolau, L., 2009. New Zealand Sauvignon blanc distinct flavor characteristis: Sensory, chemical, and consumeraspects. Am. J. Enol. Vitic. 60, 1-12.

Macheix, J. J., Sapis, J. C., Fleuriet, A. & Lee, C. Y., 1991. Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. Crit. Rev. Food Sci. Nutr. 30, 441-486.

Maggu, M., Winz, R., Kilmartin, P. A., Trought, M. C. T. & Nicolau, L., 2007. Effect of skin contact and pressure on the composition of Sauvignon blanc must. J. Agric. Food Chem. 55, 10281-10288.

Makhotkina, O. & Kilmartin, P. A., 2009. Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: Cyclic voltammetry. J. Electroanal. Chem. 633, 165-174.

Marais, J., 1983. Terpenes in the aroma of grapes and wines: A review. S. Afr. J. Enol. Vitic. 4 (2), 49-60.

Marais, J., 1994. Sauvignon blanc Cultivar Aroma - A Review. South African Journal of Enology and Viticulture. 15, 41-45.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. S. Afr. J. Enol. Vitic. 19 (1), 10-16.

Marais, J., Hunter, J. J. & Haasbroek, P. D., 1999. Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. S. Afr. J. Enol. Vitic. 20, 19-30.

Marais, J. & Pool, H. J., 1980. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. Vitis. 19, 151-164.

Marais, J. & Rapp, A., 1988. Effect of skin-contact time and temperature on juice and wine composition and wine quality. S. Afr. J. Enol. Vitic. 9, 22-30.

Marais, J., Van Wyk, C. J. & Rapp, A., 1992. Effect of storage time, temperature and region on the levels of 1,1,6-trimethyl-1,2-dihydronaphthalene and other volatiles, and on quality of Weisser Riesling. S. Afr. J. Enol. Vitic. 13, 33-44.

Margalit, Y., 1997. Concepts in wine chemistry (2). The wine appreciation guild, San Francisco.

Marshall, M. R., Kim, J. & Wei, C., 2000. Enzymatic Browning in Fruits.

Masneuf-Pomarède, I., Mansour, C., Murat, M.-L., Tominaga, T. & Dubourdieu, D., 2006. Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines. Int. J. Food Microbiol. 108, 385-390.

Mauricio, J. C., Moreno, J., Luis, Z., Ortega, J. M. & Medina, M., 1997. The effects of grape must fermentation conditions on volatile alcohols and esters formed by *Saccharomyces cerevisiae*. J. Sci. Food Agric. 75, 155-160.

Moio, L., Ugliano, M., Genovese, A., Gambuti, A., Pessina, R. & Piombino, P., 2004. Effect of antioxidant protection of must on volatile compounds and aroma shelf life of Falanghina (*Vitis vinifera* L.) wine. J. Agric. Food Chem. 52, 891-897.

Monagas, M., Bartolome, B. & Gomez-Cordoves, C., 2005. Updated knowledge about the presence of phenolic compounds in wine. Critical reviews in food science and nutrition. 45, 85-118.

Murat, M., Masneuf, I., Darriet, I., Lavigne, V., Tominaga, T. & Dubourdieu, D., 2001a. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. Am. J. Enol. Vitic. 52 (2)

Murat, M. L., Tominaga, T. & Dubourdieu, D., 2001b. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rosé wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. 49 (11), 5412-5417.

Nikolantonaki, M., Chichuc, I., Teissedre., P. L. & Darriet, P., 2010. Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. Analytica Chimica Acta. 660, 102-109.

Nordström, K., 1965. Possible control of volatile ester formation in brewing. In: (ed). Proc. Proc. Europ. Brew. Conv., Stockholm. pp. 195-208

Nykänen, L., 1986. Formation and occurance of flavor compounds in wine and distilled alsoholic beverages. Am. J. Enol. Vitic. 37 (1), 84-96.

Ough, C. S., 1985. Some effects of temperature and SO_2 on wine during simulated transport or storage. Am. J. Enol. Vitic. 36 (1), 18-22.

Papadopoulou, D. & Roussis, I. G., 2001. Inhibition of the decline of linalool and α-terpineol in muscat wines by glutathione and N-acetyl-cysteine. It. J. Food Sci. 13, 413-419.

Papadopoulou, D. & Roussis, I. G., 2008. Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by glutathione and N-acetylcysteine. International Journal of Food Science and Technology. 43, 1053-1057.

Patel, P., Herbst-Johnstone, M., Lee, S. A., Gardner, R. C., Weaver, R., Nicolau, L. & Kilmartin, P. A., 2010. Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of Sauvignon blanc microferments. J. Agric. Food Chem. 58, 7280-7288.

Peng, Z., Duncan, B., Pocock, K. F. & Sefton, M. A., 1998. The effect of ascorbic acid on oxidative rowning of white wines and model wines. Australian Journal of Grape and Wine Research. 4, 127-135.

Peynaud, E., 1984. Knowing and Making Wine. John Wiley & Sons

Peyrot Des Ganchos, C., Tominaga, T. & Dubourdieu, D., 2000. Measuring the aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc grapes by assaying S-cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma. J. Agric. Food Chem. 48 (8), 3387-3391.

Peyrot Des Ganchos, C., Tominaga, T. & Dubourdieu, D., 2002a. Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. J. Agric. Food Chem. 50, 4076-4079.

Peyrot Des Ganchos, C., Tominaga, T. & Dubourdieu, D., 2002b. Localization of S-cysteine conjugates in the berry: Effect of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc must. Am. J. Enol. Vitic. 53 (2), 144-146.

Peyrot Des Ganchos, C., Van Leewin, C., Tominaga, T., Soyer, J.-P., Gaudillere, J.-P. & Dubourdieu, D., 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L. cv. Sauvignon blanc in field conditions. J. Agric. Food Chem. 85, 73-85.

Radler, F., 1993. Yeast-metabolism of organic acids. In*:* Fleet, G. H. (eds). Wine microbiology and biotechnology, Hardwood academic publishers, Chur, Switzerland. pp. 165-182.

Ramey, D. D. & Ough, C. S., 1980. Volatile ester hydrolysis or formation during storage of model solutions and wines. J. Agric. Food Chem. 28, 928-934.

Rapp, A. & Mandery, H., 1986. Wine Aroma. Experimentia. 42, 873-884.

Rauhut, D., 1993. Yeasts-production of sulfur compounds. In*:* Fleet, G. H. (eds). Wine microbiology and biotechnology, Hardwood Academic, Chur, Switzerland. pp. 183-223.

Ribéreau-Gayon, P., 1965. C.R. Acad. Sciences. 260, 341.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A., 2006a. Handbook of Enology The microbiology of wine and vinifications (2). 1. John Wiley & Sons Ltd, Chichester.

Ribéreau-Gayon, P., Glories, Y., Dubourdieu, D. & Maujean, A., 1998, 2004b. Traite d'oenologie. Chimie du vin. Stabilisation et traitements., Dunod Paris.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D., 2006b. Handbook of Enology The chemistry of wine stabilization and treatments (2). 2. John Wiley & Sons Ltd, Chichester.

Roland, A., Schneider, R., Guerneve, C. L., Razungles, A. & Cavelier, F., 2010a. Identification and quantification by LC-MS/MS of a new precursor of 3-mercaptohexan-1-ol (3MH) using stable isotope dilution assay: elements for understanding the 3MH production in wine. Food Chem. DOI:10.1016/j.foodchem.2009.12.095,

Roland, A., Vialaret, J., Razungles, A., Rigou, P. & Schneider, R., 2010b. Evolution of *S*-Cysteinylated and *S*-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. J. Am. Chem. Soc. 58, 4406-4413.

Roujou De Boubée, D., Cumsille, A. M., Pons, M. & Dubourdieu, D., 2002. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractibility during vinification. Am. J. Enol. Vitic. 53 (1), 1-5.

Roussis, I. G., Lambropoulos, I. & Papadopoulou, D., 2005. Inhibition of the decline of volatile esters and terpenols during oxidative storage of Muscat-white and Xinomavro-red wine by caffeic acid and N-acetyl-cysteine. J. Agric. Food Chem. 93, 485-492.

Roussis, I. G., Lambropoulos, I. & Tzimas, P., 2007. Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. Am. J. Enol. Vitic. 58 (2), 274-278.

Salgeus, M., Cheynier, V., Gunata, Z. & Wulde, R., 1986. Oxidation of grape juice 2-*S*-glutathionyl caffeoyl tartaric acid by *Botrytis cinerea* laccase and characterization of new substance 2,5-di-*S*-glutathionyl-caffeoyl tartaric acid. J. Food Sci. 51, 1191-1194.

Sayavedra-Soto, L. A. & Montgomery, M. W., 1986. Inhibition of polyphenoloxidase by sulfite. J. Food Sci. 51, 1531-1536.

Schneider, V., 1998. Must hyperoxidation: A review. Am. J. Enol. Vitic. 49 (1), 65-73.

Sergianitis, S. & Roussis, I. G., 2007. Protection of volatile esters and terpenes during storage of a white wine and a model wine medium by a mixture of N-acetyl-cysteine and caffeic acid. Eur. Food. Res. Technol.

Silva Ferreira, A. C., Hogg, T. & De Pinho, P. G., 2003. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J. Agric. Food Chem. 51

Simpson, R. F., 1978. Aroma and compositional changes in wine with oxidation, storage and ageing. Vitis. 17,274-287.

Singleton, V. L., 1987. Oxygen with phenols and related reactions in must, wines and model systems: observations and practical implications. Am. J. Enol. Vitic. 38, 69-77.

Singleton, V. L., Salgues, J., Zaya, J. & Trousdale, E., 1985. Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. Am. J. Enol. Vitic. 36, 50-56.

Singleton, V. L., Zaya, J., Trousdale, E. & Salgues, M., 1980. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. Am. J. Enol. Vitic. 31 (1), 14-20.

Singleton, V. L., Zaya, J., Trousdale, E. & Salgues, M., 1984. Caftaric acid in grapes and conversion to a reaction product during processing. Vitis. 23, 113-120.

Soles, R. M., Ough, C. S. & Kunkee, R. E., 1982. Ester concentration differences in wine fermented by various species and strains of yeasts. Am. J. Enol. Vitic. 33, 94-98.

Subileau, M., Schneider, R., Salmon, J. M. & Degryse, E., 2008. New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon blanc wines: Cys-3MH and (E)-Hexen-2-al are not the major precursor. J. Agric. Food Chem. 56, 9230-9235.

Swiegers, J. H., Capone, D. L., Pardon, K. H., Elsey, G. M., Sefton, M. A., Francis, I. L. & Pretorius, I. S., 2007. Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. Yeast. 24, 561-574.

Swiegers, J. H., Francis, I. L., Herderich, M. J. & Pretorius, I. S., 2006. Meeting consumer expectations through management in vineyard and winery: the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon blanc wine. Austral. N.Z. Wine Ind. J. 21, 34-42.

Swiegers, J. H., Willmott, R., Hill-Ling, A., Capone, D. L., Pardon, K. H., Elsey, G. M., Howell, K. S., De Barros Lopes, M. A., Sefton, M. A., Lilly, M. & Pretorius, I. S., 2005. Modulation of volatile thiol and ester aromas by modified wine yeast. In: (ed). Proc. Weurman flavour research symposium. 21-24 June 2005, Roskilde, Denmark.

Thurston, P. A., Taylor, R. & Ahvenainen, J., 1981. Effects of linoleic acid supplements on the synthesis by yeast of lipids and acetate esters. Journal of the Institute of Brewing. 87, 92-95.

Tominaga, T., Baltenweck-Guyot, R., Peyrot Des Gachons, C. & Dubourdieu, D., 2000. Contribution of volatile thiols to the aromas of white wines made from several Vitis vinifera grape varieties. Am. J. Enol. Vitic. 51 (2)

Tominaga, T., Darriet, P. & Dubourdieu, D., 1996. Identification of 3-mercaptohexyl acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor. Vitis. 35, 207-210.

Tominaga, T., Furrer, A., Henry, R. & Dubourdieu, D., 1998a. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. Flavour and Fragrance J. 13, 159-162.

Tominaga, T., Masneuf, I. & Dubourdieu, D., 2004. Powerful aromatic volatile thiols in wines made from several *Vitis vinifera* L. Cv. Sauvignon blanc. In: (ed). Proc. ACS Symp. Ser.

Tominaga, T., Murat, M.-L. & Dubourdieu, D., 1998. Development of a method analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. J. Agric. Food Chem. 46, 1044-1048.

Tominaga, T., Niclass, Y., Frerot, E. & Dubourdieu, D., 2006. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (Var. Sauvignon blanc and Semillon). J. Agric. Food Chem. 54, 7251-7255.

Tominaga, T., Peyrot Des Gachons, C. & Dubourdieu, D., 1998b. A new type of flavour precursors in Vitis vinifera L. cv. Sauvignon blanc: S-cysteine conjugates. J. Agric. Food Chem. 46, 5215-5219.

Vaimakis, V. & Roussis, I. G., 1996. Must oxygenation together with glutathione addition in the oxidation of white wine. Food Chemistry. 57, 419-422.

Valero, E., Millan, C. & Ortega, J. M., 2001. Influence of oxygen addition during growth phase on the biosynthesis of lipids in *Saccharomyces cerevisiae* (M330-9) in enological fermentations. J. Biosc. Bioeng. 92, 33-38.

Valero, E., Millan, C. & Ortega, J. M., 2002. Higher alcohols and esters production by *Saccharomyces cerevisiae*. Influence of the initial oxygenation of the grape must. Food Chem. 78, 57-61.

Van Wyk, C. J., Louw, A. & Rabie, I. M., 1996. The effect of reductive wine making conditions on wine quality and composition. In: Lemperle, E., H. Trogus & P. Figlestahler (ed). Proc. 11th Int. Oenol. Symp. 3-5 June 1996. Sopron, Hungary. pp. 180-200

Versini, G., Inama, S. & Sartori, G., 1981. A capillary column gaschromatographic research into the terpene constituents of "Riesling Romano" (Rhine Riesling) wine from Trentino Alto Adige: Their distribution within berries, their passage into the must and their presence in the wine according to different wine-making procedures. Organoleptic considerations. Vini Ital. XXIII, 189-211.

Versini, G., Rapp, A. & Dalla Serra, A., 1992. Considerations about the presence of free and bound p-menth-1-enediols in grape products Schreier, P. & Winterhalter, P. (eds.), Wurzburg, Germany.

Vrhovšek, U., 1998. Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. J. Agric. Food Chem. 46, 4203-4208.

Waterhouse, A. L. & Laurie, V. F., 2006. Oxidation of wine phenolics: A critical evaluation and hypotheses. Am. J. Enol. Vitic. 57 (3), 306-313.

Weinges, K. & Piretti, M. V., 1972. Annali Chimica. 62, 45-46.

White, B. B. & Ough, C. S., 1973. Oxygen uptake studies on grape juice. Am. J. Enol. Vitic. 24, 148-152.

Wilderandt, H. L. & Singleton, V. L., 1974. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. Am. J. Enol. Vitic. 25, 119-126.

Williams, P. J., Strauss, C. R. & Wilson, B., 1980. Hydroxylated linalool derivatives as precursors of volatile monoterpenes of muscat grapes. J. Agric. Food Chem. 28

Wolf, A. E., Dietz, K. J. & Schroder, P., 1996. Degradation of glutathione S-conjugates by carboxypeptidase in the plan vacuole. FEBS Lett. 284, 31-34.

Youngblood, M. P., 1986. Kinetics and mechanism of the addition of sulfite to *p*-benzoquinone. J. Org. Chem. 51, 1981-1985.

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H. & Nury, F. S., 1995. Wine analysis and production. Chapman & Hall, New York.

Chapter 3

Research results

Effect of must oxygenation and sulphur dioxide addition on polyphenols, glutathione and certain aroma compounds in Sauvignon blanc

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3. Effect of must oxygenation and sulphur dioxide addition on polyphenols, glutathione and certain aroma compounds in Sauvignon blanc

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ABSTRACT

Winemakers in South Africa often use reductive treatments, such as sulphur dioxide and ascorbic acid additions and inert gases, on Sauvignon blanc juice to protect flavour compounds in the wine. However, the effect of different oxygen and sulphur dioxide additions to Sauvignon blanc must and the changes induced in the corresponding wines have not been investigated in detail. The present study evaluated how different oxygen and sulphur dioxide treatments of Sauvignon blanc must affects the levels of phenolic compounds, glutathione as well as aroma compounds such 2-methoxy-3-isobutylpyrazine (IBMP), 3-sulfanylhexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA) and 4-sulfanyl-4-methylpentan-2-one (4SMP). We found that wine made from oxidised juice without sulphur dioxide protection contained significantly lower levels of glutathione, phenolics, 3SH and 3SHA. However, when sulphur dioxide was present, 3SH and 3SHA levels did not decrease even when oxygen was added. Polyphenol and glutathione oxidation was also prevented in the presence of sulphur dioxide. Levels of 4SMP and IBMP did not undergo a significant decline when submitted to oxidation and therefore do not seem as sensitive towards oxidation.

KEYWORDS: Sauvignon blanc; wine aroma; volatile thiols; methoxypyrazines; glutathione; polyphenols; oxygen; sulphur dioxide

3.1 INRODUCTION

Sauvignon blanc grapes often produce wines with highly distinctive sensory characteristics. These sensory characteristics have been reported to be “green” flavours depicting vegetative, grassy, green pepper and capsicum, while “tropical” can be described as passion fruit, grapefruit, gooseberry and citrus (*1, 2*). During winemaking, Sauvignon blanc musts are often treated very reductively in South Africa as to preserve these tropical flavours. Additions of sulphur dioxide and ascorbic acid to healthy grapes with the exclusion of air and the application of moderate temperatures, has been known to

result in more fruity and fresh wines, while wines made less reductively often experienced a loss of varietal aroma with the formation of off-odours (*3, 4*).

The vegetative or "green" character can be mainly attributed to the methoxypyrazines, 2-methoxy-3-isobutylpyrazine (IBMP) and 2-methoxy-3-isopropylpyrazine (IPMP) of which IBMP are the most important contributing specifically to green pepper, capsicum, grassy and earthy odours (*1*). These compounds are often found in Sauvignon blanc wines in the range of 0.5-50 ng/L (*5, 6*) and have an extremely low odour threshold value of 2 ng/L (*7*). The pyrazine concentration normally decreases during ripening, with their maximum concentration found at veriason (*5*).

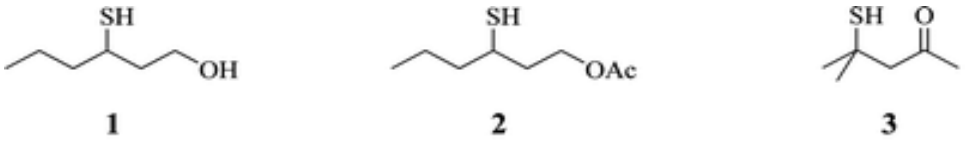

Figure 1. Structures of 3-sulfanylhexan-1-ol (3SH) (**1**); 3-sulfanylhexyl acetate (3SHA) (**2**); 4-sulfanyl-4-methylpentan-2-one (4SMP) (**3**)

Some volatile thiols (Figure 1) responsible for the fruity or tropical organoleptic flavours are 4-sulfanyl-4-methylpentan-2-one (4SMP) (*8*), reminiscent of box tree, passion fruit, broom and black current; and 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) (*9, 10*), responsible for the passion fruit, grapefruit and citrus aroma found in Sauvignon blanc wines. Perception thresholds for these compounds in model wine are 0.8 ng/L, 60 ng/L and 4.2 ng/L respectively (*11, 12*) and have been reported in Sauvignon blanc wines from France and New Zealand in the range of 4-40 ng/L, 200-18000 ng/L and 0-2500 ng/L respectively (*13, 14*). 4SMP and 3SH are almost non-existent in the grapes and are released by the yeast during fermentation from precursors of which the cysteinylated [S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) and S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4SMP)] and glutathionylated [S-3-(hexan-1-ol)-glutathione (Glut-3SH) and S-4-(4-methylpentan-2-one)-glutathione (Glut-4SMP)] precursors have been identified, although these precursors only account for a fraction of the total amount of thiols found in the wine (*8, 15-17, 53*). 3SHA are formed from the acetylation of 3SH by the yeast during fermentation (*18*). The cysteinylated and glutathionylated precursors of the aromatic thiols have stable C-S bonds, rendering them not sensitive towards oxidation (*19*). The precursor concentration should in theory stay intact during oxidative handling of the must, but in fact Glut-3SH concentrations were found to increase in the presence of sufficient oxygen, glutathione and (*E*)-2-hexenal (*19*). This increase is suspected to occur due to the Michael addition of the latter two compounds.

The degradation of the volatile thiols during wine aging has been well reported. Thiols are highly reactive compounds that oxidise easily in the presence of oxygen and metal ions such as iron and copper to form a disulphide (*21, 22*). Thiols are nucleophiles and capable of addition to electrophiles especially in the presence of limited SO_2 concentrations (*23-25*). This is a nucleophilic, acid-catalyzed substitution

reaction with phenolic compounds such as *trans*-caftaric acid (*26*). Decrease can also be ascribed to the Micheal addition (*27*) of the thiol with reactive species, such as *o*-quinones, originating from polyphenol oxidation in the presence of iron and dissolved oxygen (*23, 28*). Work by Nikolantonaki *et al*., 2010a showed differences in the affinity of *o*-quinones for certain thiols, but this work done by them was performed in a model wine solution and is therefore not a true representative of the complex reactions possibly taking place in wine.

Previous research clearly stated the importance of reductive handling of the wine (*29*). In the wine, the volatile thiols are present and susceptible to oxidation. However, there seems to be a lack of information as to why the juice needs to be treated reductively before fermentation as the thiols have not yet been formed at this stage of the winemaking process. In grape juice, rapid enzymatic oxidation takes place in the presence of polyphenol oxidase (PPO) and oxygen (*30*). The main hydroxycinnamic acid normally present, *trans*-caftaric acid, is degraded faster than (+)-catechin and (-)-epicatechin to form the corresponding *o*-quinone (*31*). The formed *o*-quinone is chemically unstable and can condense with other phenolic compounds to form yellow and brown pigments (*30*). The *o*-quinone can also combine with thiol containing compounds, such as glutathione (GSH), forming the grape reaction product (GRP) (*27, 32*). The oxidation enzymes can be inhibited by SO_2 and as long as sufficient GSH and SO_2 are available, the enzyme activity will decrease and the GRP will prevent the *o*-quinone from participating in the formation of pigments (*33*). In grape must, added sulfur dioxide acts in different ways to inactivate the mechanism of flavonoid precipitation in must. It inhibits and destroys tyrosinase; total activity decrease is 75 % to 90 % when 50 mg/L SO_2 are added (*34*).

The aim of this study was thus to investigate the effect of juice oxidation and SO_2 additions on the chemical profiles of Sauvignon blanc wines. Recent studies have investigated the effect of oxidation on thiol precursor concentrations (*19*), effect of free run and press juice on thiols produced (*35*) or the interactions between volatile thiols and oxidised phenolics in a model wine media (*31*). In this study the effect of pre-fermentative oxygen and SO_2 addition to Sauvignon blanc must on the volatile thiol and methoxypyrazine composition of the resulting wines was shown.

3.2 MATERIALS AND METHODS

3.2.1 Juice and Winemaking Treatments

Juice samples were obtained from two different cellars (cellar 1 and cellar 2) during the 2009 harvest. The grapes were all grown according to standard viticultural practices in South Africa and picked by hand when ripe. Grapes from cellar 1 (juice and wine 1) had a sugar level of 23 °Brix, titratable acidity of 6 g/L and pH of 3.2. Grapes from cellar 2 (juice and wine 2) had a sugar level of 21.8 °Brix, titratable acidity of 8.81 g/L and pH of 3.31.

Grapes were handled according to the respective wineries standard practices. Grapes were crushed and pressed on the estate and procedures, equipment and additions varied to some extent between wineries. Sulphur dioxide was added at 30 mg/L by the winemakers during crushing. Juice 2 received ascorbic acid and four hours skin contact before pressing; while juice 1 was pressed immediately after crushing. Both sets of grapes were pressed hyper-reductively using Bucher Inertys® which excludes air during pressing by replacing it with nitrogen. Juice 1 was obtained as the pressing pressure reached 1 atmosphere. The free and total SO_2 concentrations of juice 1 were 1 mg/L and 9 mg/L respectively (tested by an accredited commercial laboratory using the aspiration method). Juice 2 was a mixture of juice ranging from free run up to 1 atmosphere pressed juice with free SO_2, total SO_2 and ascorbic acid (tested by an accredited commercial laboratory using an automated enzymatic procedure) concentrations of 4 mg/L, 18 mg/L and 30 mg/L respectively. The juice samples collected were intended to contain a pressed juice fraction. The reasons for this was to have a higher concentration of phenolics to amplify the oxidation effect and to have low SO_2 values in the juice (pressed juice normally contains less added SO_2 than free run juice).

Four 20 litre cylinders, filled with CO_2 gas (Afrox SA), were used to collect the juice directly from the hyperreductive press. The juice was divided into 4.5 litre glass bottles which had been previously sparged with CO_2 gas until inert atmospherewas reached, corresponding to O_2 concentration below 1%. Oxygen concentration was checked using an Oxi 330i hand held oxygen meter with a cell-ox 325 probe (Wissenschaftlich-Technische Werkstätten). All juices were treated with 0 or 60 mg/L SO_2 additions and 0 or 4 mg/L O_2 additions. In treatments where no O_2 was added the O_2 levels were kept <0.5 mg/L. The different treatments and abbreviations which will be used in this article can be seen in Table 1.

Table 1: Code and description of different oxygen and SO_2 treatments in Sauvignon blanc must

Code	**Treatment**	**Oxygen concentrations in must**	**SO_2 additions to must**
A	$-SO_2$ / $-O_2$	<0.5 mg/L	0 mg/L
B	$+SO_2$ / $-O_2$	<0.5 mg/L	60 mg/L
C	$-SO_2$ / $+O_2$	4 mg/L	0 mg/L
D	$+SO_2$ / $+O_2$	4 mg/L	60 mg/L

In the relevant treatments, the SO_2 was first added to the bottle which was then filled with juice. Oxygen levels were achieved by racking the juice into a plastic 20 litre bucket to encourage O_2 pickup with continuous measurement of the oxygen until the required values was reached. Dissolved oxygen measurement was done using the Oxi 330i. Pectolytic enzyme (Rapidase® Vino Super, DSM Oenology) was added to the juice, the bottles sealed with plastic screw caps and parafilm and settled for one day at 15 °C and the following day at 4 °C. After the two days, about 3.5 litres of the juice was racked from the grape lees under CO_2 pressure into another 4.5 litre glass bottle (which was also previously filled with

CO_2). Oxygen measurement was repeated just before inoculation and at this stage treatments A, B and C had a dissolved oxygen concentration in the range of 0.2-0.5 mg/L while treatment D had levels in the range of 1-1.6 mg/L for both cellars. All juices were inoculated with rehydrated *Saccharomyces cerevisiae* VIN 7 (Anchor Yeast Biotechnologies) at 0.3 g/L according to the supplier's recommendations and fermentations were performed at 15 °C. On the third day of fermentation, 0.75 g/L diammonium phosphate was added to the fermenting must. The escape of CO_2 during fermentation maintained an anaerobic environment. The progress of fermentation was recorded by weighing the glass bottles daily. After mass loss stopped (21 days), free SO_2 values were adjusted to 30 mg/L and the juice was cold stabilized at -4 °C for 7 days. Before bottling, the free SO_2 was adjusted once again to 30 mg/L. The wine was racked with CO_2, bottled in green 750 ml wine bottles under CO_2 gas, sealed with screw tops and stored upright at 4 °C. All treatments in the two juices were performed in triplicate and the results reported are the means of the three trials.

3.2.2 Sampling procedure

Juice samples were taken from the settled juice for the analyses of glutathione, phenols and methoxypyrazines. This was done by transferring the required volume into plastic sampling bottles using CO_2 pressure. SO_2 (1000 mg/L) and a freshly prepared solution of ascorbic acid (500 mg/L), was added to the sample bottles before filling it with juice. These high concentrations should completely inhibit any residual phenolic oxidase or laccase activity (*36*). CO_2 was blown in the bottles before the juice transfer and the headspace was also filled with CO_2 before closure. The samples were then frozen at -20 °C until analyses could be done. Wine samples were taken just before bottling for the analyses of glutathione, phenols, methoxypyrazines and volatile thiols. Samples were drawn with the same procedure as with the juice, but no SO_2 or ascorbic acid additions were made. The free SO_2 content of the wine was adjusted to 30 mg/L in preparations for bottling and was thus sufficient to protect the wine samples.

3.2.3 Glutathione analyses

Glutathione concentrations in the must and wine were analysed with the liquid chromatography mass spectrometry (LC-MS/MS) method as described by Du Toit *et al.,* 2007 (*36*). A Waters API Quattro microtriple quadropole mass spectrometer with a 2690 Alliance HPLC was used for the glutathione analyses. Separation was achieved using a Waters Atlantis C18 column (2.1 mm × 150 mm × 3 µm). Sample preparation was done by centrifuging the sulphited juice samples and diluting it 4 times using Milli-Q-Water® before injection. In order to remove alcohol, which interferes glutathione analyses, wine samples were submitted to heat (42°C) and reduced pressure for about 10 minutes using a rotovapor. The removed alcohol from the sample was replaced with Milli-Q-Water®. Juice and wine samples were placed in dark coloured vials, filled with nitrogen gas to protect the sample from oxidation, and injected into the LC-MS/MS.

3.2.4 Phenolics analyses

Reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Hewlett Packard Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Hewlett Packard, Waldbronn, Germany). Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100Å, 150 × 4.6 mm, 3 µm) from Polymer Laboratories (Ltd) (Shropshire, UK) protected with a guard cartridge (PLRP-S, 10 × 4.6 mm) (Polymer Laboratories (Ltd), Shropshire, UK) with the same packing material. The following mobile phases were used: solvent A, containing de-ionised water with 1.5 % v/v *o*-phosphoric acid (Merck) and solvent B consisting of 80% acetonitrile (Sigma) with 20 % of solvent A. A linear gradient was used from 0 min, A 94%, B 6%; to 73 min, A 69%, B 31%; to 78, A 38%, B 62%, staying constant for 8 min to 86 min and then back to the starting conditions in 4 min to 90 min, A 94%, B 6%. A flow rate of 1 ml/min was used and a column temperature of 35^{o}C. This was adapted from the method of Peng *et al.,* (2002). Phenols were quantified using external standards: (+)-catechin hydrate (Fluka), (-)-epicatechin (Sigma) and caffeic acid (Sigma). Monomeric flavanols were quantified at 280 nm as mg/L (+)-catechin units with a quantification limit of 1.5 mg/L, and (-)-epicatechin as (-)-epicatechin with a quantification limit of 1.5 mg/L. *Trans*-caftaric acid have a maximal absorbance at 316 nm and were quantified as mg/L caffeic acid. The samples were centrifuged at 14000rpm for 5 minutes before injection. Thereafter each sample was placed in a 1.5 ml dark glass vial and protected with nitrogen gas from oxidation. The limit of quantification was determined as the smallest area that could be accurately integrated (<3% standard deviation), or expressed as signal to noise ratio of at least 7 (*37*).

3.2.5 Volatile Thiol analyses

Analyses was done in the wine samples to quantify three volatile thiols, 3-sulfanylhexan-1-ol , 3-sulfanylhexyl acetate and 4-sulfanyl-4-methylpentan-2-one . These thiols were extracted according to the method described by Tominaga et al., 1998 (*11*) and Lund et al., 2009 (*14*). The method was slightly adjusted with some modifications as deuterated internal standards were used for the quantification of 3SH (3-sulfanyl(1-$^{2}H_2$)hexanol) and 3SHA (3-sulfanyl(1-$^{2}H_2$)hexyl acetate) (*38*). To 50 ml wine, 5 mL of 1 mM p-hydroxymercurybenzoate (p-HMB) solution and then 0.5 mL of 2 nM butylated hydroxyanisole (BHA) solution was added. After stirring the sample, deuterated labelled isotopes were added. 50 µL of a mix of 22 µM 3-sulfanyl(1-$^{2}H_2$)hexanol and 2.8 µM, 3-sulfanyl(1-$^{2}H_2$)hexyl acetate was added along with 25 µL of 2.5 nM 4-methoxy-2-methyl-2-sulfanylbutane (4M2M2SB) which was further used for the quantification of 4-sulfanyl-4-methylpentan-2-one. The wine sample was percolated on a anion exchange column before the thiols were eluted with cysteine and extracted into ethyl acetate and dichloromethane prior to concentration and manual injection of 2 µL onto an Agilent Gas Chromatograph 6890N coupled to an Agilent 5973 mass-selective detector (Agilent, Santa Clara, CA). The thiols were separated on a HP-Innowax (60 m × 252 µm × 0.25 µm) column with Helium as carrier

gas at 112 kPa and a flow of 23.8 mL/min and an oven temperature ramping from 50 to 250°C for a 64.3-min run. Standard curves were obtained by adding increased quantities of the three thiols to a Sauvignon blanc wine (220.4 to 3305.9 ng/L 3SH; 45 to 675 ng/L 3SHA; 4.1 to 62 ng/L 4SMP). The regression equation obtained for 3SH was y = 1057x -406.4 with R^2 = 0.995, for 3SHA it was y = 546.2x - 85.10 with R^2 = 0.991 and for 4SMP y =1061x - 24.33 with R^2 = 0.985. All of the samples were analyzed in duplicate.

3.2.6 Methoxypyrazine analyses

The 2-methoxy-3-isobutylpyrazine (IBMP) and 2-methoxy-3-isopropylpyrazine (IPMP) concentrations were determined using an automated head space solid-phase micro-extraction (HS-SPME) technique (*39*). Standard curves for quantitative analysis were generated using the deuterated internal standard, 2-methoxy-3-([1,1-$^2H_3$]isobutyl)pyrazine, as well as the non-deuterated 2-methoxy-3-isobutylpyrazine and 2-methoxy-3-isopropylpyrazine and was obtained from a commercial supplier (Sigma-Aldrich).

Sample preparation involved pipetting 6 ml of wine into 24 ml Milli-Q-Water® (i.e., a 5-fold dilution) to a total of 30 ml. To this diluted wine mixture, 37.5 µL of a 2-methoxy-3-([1,1-$^2H_3$]isobutyl)pyrazine solution at 1.01 mg/L and 37.5 µL of a 2-methoxy-3-methylpyrazine solution at 1.01 mg/L were added as internal standards. 8 mL of this solution was then pipetted into a 20 ml amber SPME sample vial containing 3 g crystalline sodium chloride and a magnetic glass stir bar. Immediately after that, 2 ml of 4 M sodium hydroxide (NaOH) was added to the tube. The vial was then gently purged with argon gas and then immediately closed with a silicone screw cap. Samples were put onto a Gertsel multi-purpose sampler, after which they were transferred to a Gerstel agitator/stirrer for 5 minutes incubation at 45 ^oC. The sample was held on the incubator for 5 minutes at 40 ^oC. The sample was agitated at 350 rpm for 50 seconds, then no agitation occurred for 3 seconds, after which agitation continued for 50 seconds intervals for the entire incubation time. The sample was extracted using automated HS-SPME with a multi fibre consisting of CAR-PDMS-DVB combination (50/30 µm × 2 cm). The vial was penetrated down 0.31 mm with the fibre being exposed to the headspace for 40 minutes before being desorbed for 20 min. Analysis was carried out on an Agilent 7890 GC that is coupled with a 5975C inert XL mass spectrometer detector. The column used is a tandem column composed of an HP-1MS column (30 m × 0.32 mm × 0.25 µm) and a HP-Innowax fused silica capillary column (30 m × 0.32 mm × 0.25 µm). Temperature profile of the column was as such: the temperature was kept at 60 ^oC for 5 minutes, after which it was raised to 170 ^oC at 4 ^oC/minute. It was then raised to 230 ^oC at 40 ^oC/minute (held for 20 minutes). The temperature was then lowered back to 60 ^oC at 40 ^oC/minute. Injection was in splitless mode with the front inlet kept at 250 ^oC and 28 kPa. During elution of the methoxypyrazines the GC-MS was switched to single ion monitoring (SIM) mode and tuned to measure *m/z* values of 137 and 152 for IPMP, 127 and 154 for 2-methoxy-3-([1,1-$^2H_3$]isobutyl)pyrazine and 124 and 151 for IBMP, respectively. The *m/z* ratios of 137/127 and 124/127 were used to quantify the IPMP and IBMP wine concentrations

respectively. The standard curve was prepared by adding increasing quantities of IBMP (from 2.1 to 51.9 ng/L) and IPMP (from 2.0 to 51.2 ng/L) to a Sauvignon blanc wine to obtain eight different concentrations. The regression equation obtained was y= 347.5x + 0.6414 with r^2 = 0.9984 for IBMP and y= 170.02x - 1.0774 with r^2= 0.9981 for IPMP. Relative standard deviations of 5.40% and 6.47% were obtained for IBMP and IPMP, respectively, by assessing 5 samples of the same wine.

3.2.7 Data Analyses

All analyses were done using Statistica 9. For statistical analyses one-way ANOVA's were conducted in cases where differences between treatments only were investigated. For those analyses where the effects of treatments and difference between juice and wine were included, mixed model repeated measures ANOVA's were used. Fisher least significant difference (LSD) corrections were used for post-hoc analyses. Significant differences were judged on a 5% significance level ($p<0.05$).

3.3. RESULTS AND DISCUSSION

Fermentation rates between the different treatments differed slightly, with treatments C fermenting slightly faster. All wines fermented to residual sugar levels lower than 4 g/L (results not shown).

3.3.1. Glutathione concentrations

Glutathione can occur in grape juice in concentrations ranging from 14-103 mg/L (*36, 40*) and can possibly be used as an oxidation marker due to its sensitivity to oxidation. Glutathione could also have a positive preserving effect of certain volatile aroma compounds in wine (*41*).

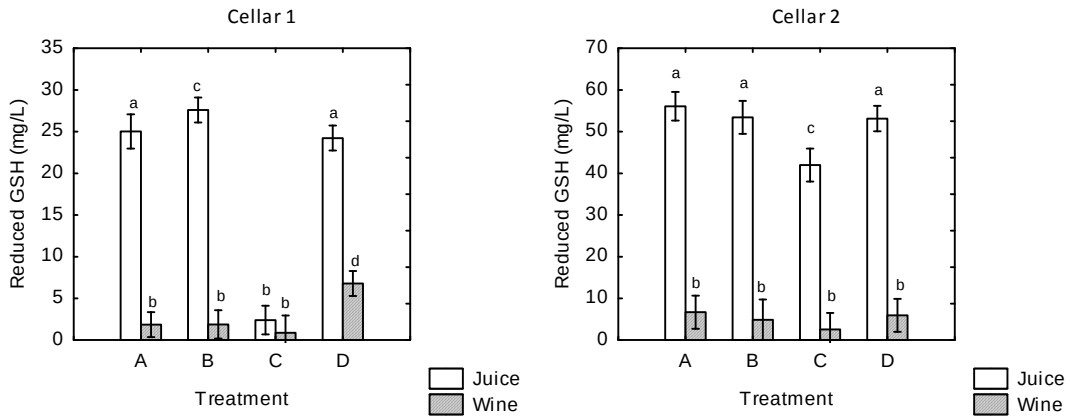

Figure 2. Reduced glutathione (GSH) concentration in juice and wine of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2/-O_2$); B ($+SO_2/-O_2$); C ($-SO_2/+O_2$); D ($+O_2/+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

In figure 2 the glutathione concentrations in grape must and the corresponding wines from the two cellars can be seen. There was little to no difference in glutathione concentrations between treatments A, B and D in both musts. The levels of reduced glutathione in the juice differed significantly where oxidation took place. The addition of oxygen without the protective effect of sulphur dioxide (treatment C) caused a severe drop in glutathione concentrations in the must. This decrease was not found in treatment D as sufficient sulphur dioxide was present to inhibit the oxidation of glutathione. Juice 2 showed the same tendency of lowered levels of glutathione with the oxidative treatment C. This decrease was not as severe as in Juice 1, although significant. This could be due to the addition of the antioxidant, ascorbic acid, by the winemaker during crushing of the grapes or the higher initial concentration of glutathione in Juice 2 when compared to Juice 1. Grapes from different origins differ in their composition of oxidising and reducing agents. Oxidising enzymes activity, of PPO and laccase can also differ depending on the maturity, origin and health of the grapes (*42*). Glutathione, with its electron-rich nucleophilic sulfanyl centre spontaneously substitutes by Micheal addition into the electrophilic centre of the *o*-quinone (formed by the oxidation of polyphenols) forming the GRP (*32*). More GRP (although not measured) would have formed in treatment C, resulting in less reduced glutathione available for further protection against oxidation and more *o*-quinones being present in the juice. Treatment D did not cause a significant decrease in glutathione concentrations indicating low oxidation activity due to the inhibition of the PPO enzyme by the presence of the antioxidant, sulphur dioxide in concentrations >50 mg/L (the concentration reported to inhibit this enzyme) (*42*).

It is clear that glutathione concentrations decrease during fermentation, possibly due to yeast metabolism. Higher levels of glutathione have been found before in wines made from reductively treated musts compared to their oxidative counterparts (*36*). However, this tendency was not observed in the present study as there were no significant differences in glutathione concentrations between the different treatments, except for those present in treatment D of juice 1. Yeast strain and initial nitrogen and glutathione content of the must can all influence the eventual concentration in the wine (*36, 43*).

3.3.2. *Trans*-caftaric acid, (+)-catechin and (-)-epicatechin concentrations

Trans-caftaric acid concentrations in Juice 1 and 2 were in line with those found by other authors (*35, 44, 45*). T*rans*-caftaric acid concentrations in Juice 1 and 2 decreased significantly, when treated with oxygen without SO_2 (treatment C). However, *trans*-caftaric acid concentrations in the other treatments in some cases did show significant differences, but not as drastic as with treatment C (Figure 3).

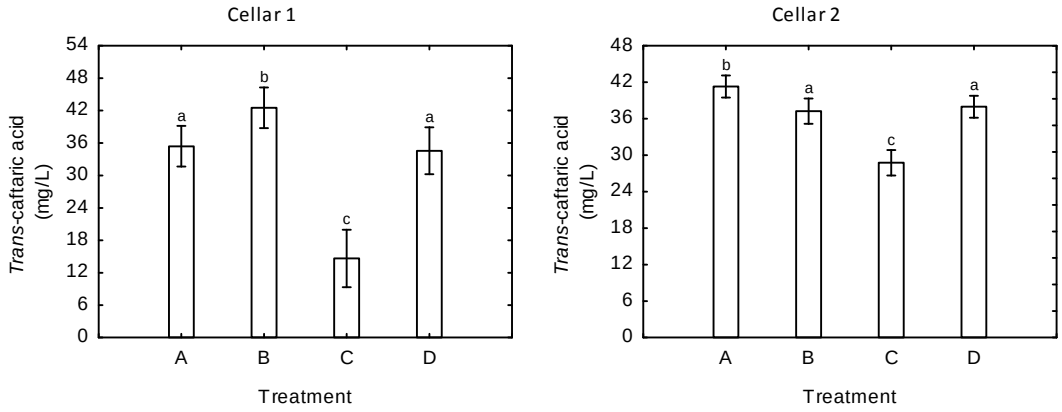

Figure 3. *Trans*-caftaric acid concentration in juice of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

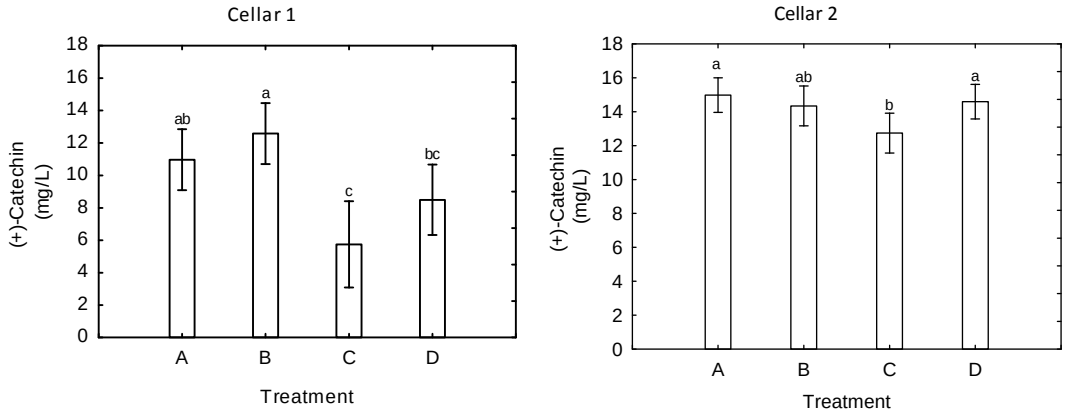

Figure 4. (+)-Catechin concentration in juice of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

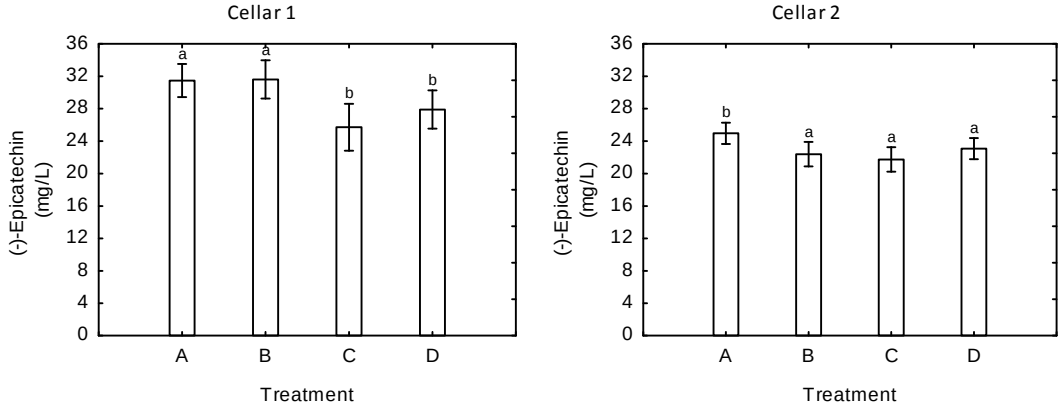

Figure 5. (-)-Epicatechin concentration in juice of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

The flavan-3-ols decreased in the same tendency as *trans*-caftaric acid with (+)-catechin experiencing a significant decrease in the oxidative treatment C of Juice 1 and 2 (Figure 4). These concentrations of the flavan-3-ols is higher than is normally found in white grape musts, due to press juice also being present (*45, 46*). Significant higher (-)-epicatechin levels (Figure 5) in juice 1 were observed in treatments A and B, with treatments C and D (despite the addition of sulphur dioxide in the latter treatment) being significantly lower. However, these decreases were not as dramatic as with *trans*-caftaric acid. (-)-Epicatechin concentrations in Juice 2 were not statistically different between the treatments except in the case of treatment A. In the case of (-)-epicatechin, the addition of SO_2 in the presence of oxygen did not seem to have had the same protective effect as with *trans*-caftaric acid. It has also been found that during chemical oxidation in a model wine solution, (-)-epicatechin had a higher reaction speed with oxygen than sulphur dioxide (*29*). The preference for specific substrates by the PPO enzyme was clearly *trans*-caftaric acid converting higher amounts of *trans*-caftaric acid to the corresponding *o*-quinone when compared to the flavan-3-ols. This confirmed work performed by Cheynier *et al.,* 1988 (*47*) where *trans*-caftaric acid was also oxidised more quickly than (+)-catechin and (-)-epicatechin in model solutions. *Trans*-caftaric acid was also found to increase the oxidation rate of some of these flavanoids, but it's *o*-quinone can also be reduced back by these flavanoid compounds (*36, 47, 48*). However, it is clear that sulphur dioxide partly or completely prevented the oxidation of *trans*-caftaric acid, (+)-catechin and (-)-epicatechin. This could be due to inhibition of the PPO by the SO_2 (*42*), the reaction of SO_2 with the formed hydrogen peroxide or reduction effect of SO_2 on the *o*-quinones, converting them back to the corresponding phenolic compound (*49*). However, the first mechanism is most likely, as chemical oxidation is a much slower process than enzymatic oxidation.

3.3.3. Volatile Thiol concentrations

Wines 1 and 2 made from treatment C both had significant lower levels 3SH and 3SHA (Figures 6 and 7).

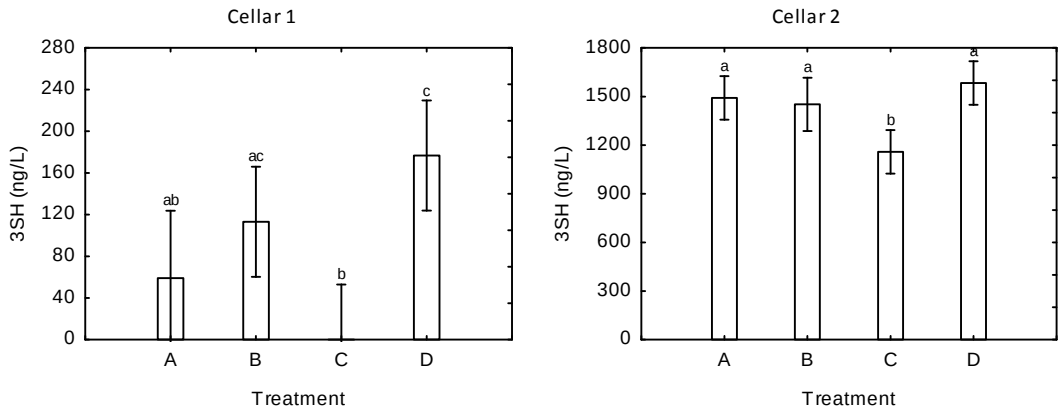

Figure 6. 3-Sulfanylhexan-1-ol (3SH) concentration in wine of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

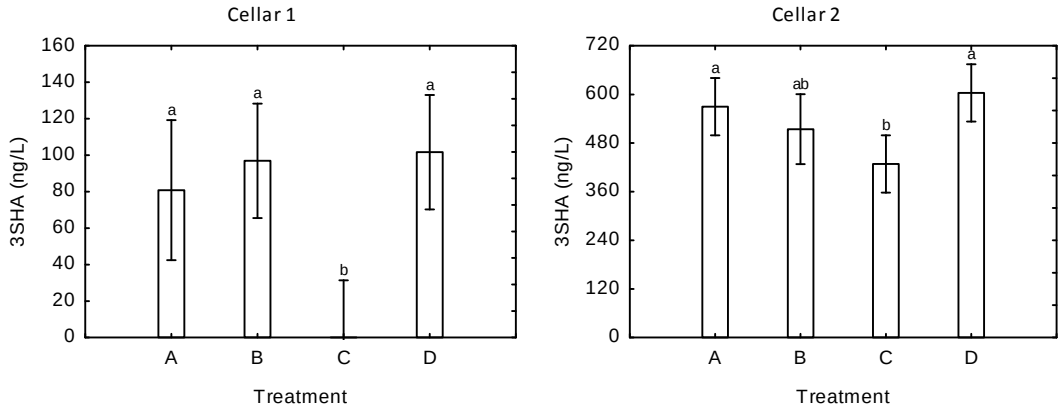

Figure 7. 3-Sulfanylhexyl acetate (3SHA) concentration in wine of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

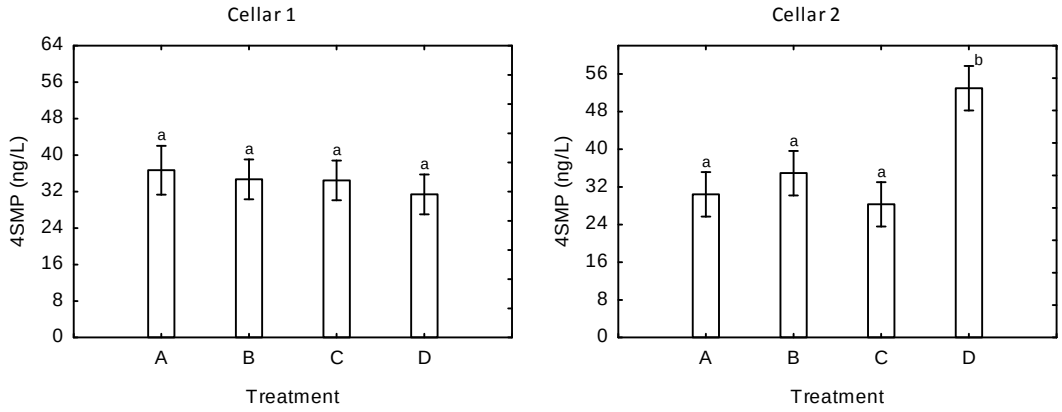

Figure 8. 4-Sulfanyl-4-methylpentan-2-one (4SMP) concentration in wine of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$ /$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

This is probably due to the reaction of the thiol formed during alcoholic fermentation with the *o*-quinones (*29*) originating from the oxidation of the must, as the thiol precursors are not sensitive to oxidation (*19*). The thiol undergoes transformation due to Michael addition with the *o*-quinone leading to loss in aroma. The thiol concentration in treatment D did not experience the same decrease as in treatment C, as the formation of the *o*-quinone in the juice and the subsequent addition reaction to the thiol in the wine was blocked by the presence of SO_2, even in the presence of oxygen. Our results in general correlated with those of Blanchard *et al*. 2004, although these experiments were performed on 3SH only in red wine.

It would seem as if only 3SH and 3SHA was drastically influenced by the oxidative treatments. 4SMP concentrations showed no significant difference between the treatments for wine 1. The oxidation sensitivity is much less than the other volatile thiols as 4SMP has a low affinity for phenols and the corresponding *o*-quinones (*29*). This difference in reactivity of the thiols can be due to the structure of

the thiol as 3SH is a secondary thiol and 4SMP is tertiary causing more steric hindrance (*29*). The levels of 4SMP also supports findings in literature stating that Glut-4SMP can be formed by addition of glutathione and mesityl oxide in oxidative conditions, however mesityl oxide has never been identified in grape must, although its hydrate has been reported in wine at a mean level of 50µg/L and in some Japanese grape varieties (*19, 50*). Wine 2 had elevated levels of 4SMP when treated with oxygen and SO_2, which cannot be explained at this stage as this contradicts previous findings. The possibility of the presence of mesityl oxide could be explored.

The effect of must oxygenation and sulphur dioxide additions on the yeast performance must also be kept in mind. Oxygen has a considerable impact on the fermentation kinetics of grape must (*36*). Must oxygenation before fermentation modifies yeast metabolism by increasing the amount of sterols produced and favouring cellular growth and viability (51).

The presence of sufficient SO_2 thus seems to protect the juice from oxidation, even when agitated with air. Pressing of berries will produce free-run juice with low oxidative potential as it has higher SO_2 concentrations, low phenol content and higher glutathione content (*45*). However, press juice obtained in the presence of air will be less protected as it has higher oxidative potential with low SO_2, low glutathione levels and high phenol concentrations. Reductive treatments of press juice would thus be favourable. Even though more cysteinylated thiol precursors are found in press juice, which might also be true for the recently identified 3SH-GSH precursor (*16, 45, 53*), higher levels of 3SH and 3SHA was found in the wines made from free-run juice (*35*). Another explanation could be due to higher concentrations of phenolics in the press juice and lower concentrations of SO_2, leading to the formation of *o*-quinones being able to bind to thiols. Reductive handling of the free-run juice, by using inert gas is therefore not critical, provided sufficient SO_2 is present. However the addition of SO_2 during grape pressing would be advisable in systems where an inert atmosphere can not be established.

3.3.4. Methoxypyrazine concentrations

The IBMP concentrations found in both musts and wine was very low compared to levels found in New Zealand (9-47 ng/L) (*14*), being just above the odour threshold (2 ng/L). Even though the aroma could easily be masked at these low concentrations, the chemistry and reactivity of the compound would probably stay the same.

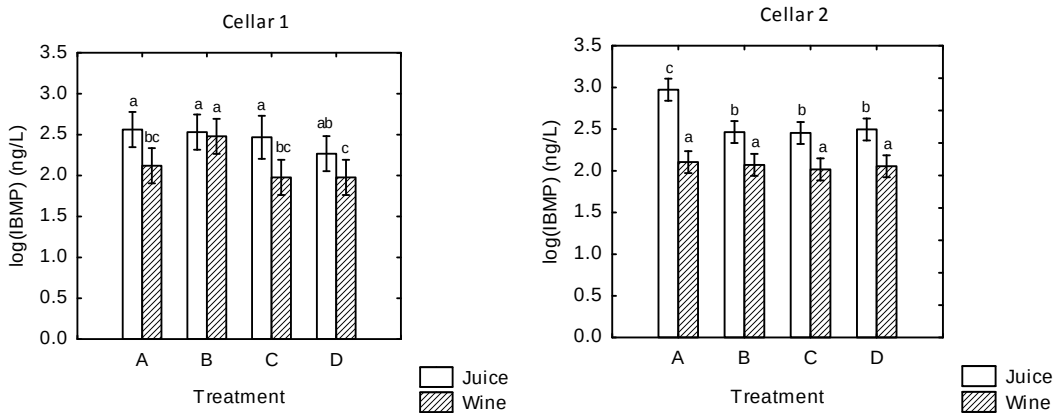

Figure 9. 2-Methoxy-3-isobutylpyrazine (IBMP) concentration in juice and wine of cellar 1 and cellar 2 undergoing different SO_2 and O_2 treatments. A ($-SO_2$ /$-O_2$); B ($+SO_2$ /$-O_2$); C ($-SO_2$ /$+O_2$); D ($+O_2$/$+SO_2$). Error bars indicate 95% confidence intervals for the means. Letters indicate significant differences on a 5% ($p<0.05$) significance level.

Juice 1 had no significant differences in the amount of IBMP present after the treatment. Oxidation did not influence the content of methoxypyrazines. It would seem as if the treatment B (no oxygen, added sulphur dioxide), had a slight preserving effect on the pyrazine resulting in higher values in the wine in this treatment. In juice 2 only treatment A had significant higher values in the juice, which we cannot explain at this stage. This elevated levels was however so minor (0.4-0.5 ng/L difference) in terms of general concentration ranges found in juice and did not show any significant differences in the resulting wine, it would probably have no sensory effect. The concentration of 2-methoxy-3-isopropylpyrazine (IPMP) was also determined (data not shown) and the same effect was observed. It could thus be concluded that the added oxygen to the juice and oxidation reactions thereafter, had no large effect on the pyrazine levels in must and wine. This tendency correlates with studies done by Marais, 1998 (*4*).

3.4 CONCLUSIONS

It has been long known that oxidation of juice can have a positive effect on the oxidative stability of the resulting wine, but can lead to decreases in varietal aroma compounds (*20, 52*), which could in general lead to lower quality wines. The present study supports the findings from Marais, 1998 (*4*) as the levels of IBMP did not decrease when submitted to an oxidative environment. Volatile thiols differed in their reactions to the oxidative treatments, but in general were protected against oxidation by the antioxidant function of SO_2. Furthermore, it seems as if the addition of oxygen to the must with the protection of sulphur dioxide could lead to an increase in the 3SH precursor, Glut-3SH, possibly resulting in higher concentrations of 3SH and 3SHA in wines (*19*). This study clearly showed the effectiveness of moderate SO_2 additions in protecting thiols from unwanted association with *o*-quinones in press juice. This study could have a significant impact on the way Sauvignon blanc musts are handled in the cellar. The enhancement of fermentation by the addition of oxygen, without preventing oxidation of phenolics,

can lead to changes in volatile thiol production. A greater understanding of the evolution of the volatile thiols during fermentation and winemaking in general is further required to be able to improve the aroma profile of Sauvignon blanc wines.

3.5 ABBREVIATIONS USED

IBMP, 2-methoxy-3-isobutylpyrazine; IPMP, 2-methoxy-3-isopropylpyrazine; 3SH, 3-sulfanylhexan-1-ol; 3SHA, 3-sulfanylhexyl acetate; 4SMP, 4-sulfanyl-4-methylpentan-2-one; Cys-3SH, S-3-(hexan-1-ol)-L-cysteine; Cys-4SMP, S-4-(4-methylpentan-2-one)-L-cysteine; Glut-3SH, S-3-(hexan-1-ol)-glutathione; Glut-4SMP, S-4-(4-methylpentan-2-one)-glutathione; GSH, glutathione; GRP, grape reaction product; PPO, polyphenol oxidase; LC-MS/MS, liquid chromatography mass spectrometry; RP-HPLC, reverse phase high performance liquid chromatography; p-HMB, p-hydroxymercurybenzoate; BHA, butylated hydroxyanisole; 4M2M2SB, 4-methoxy-2-methyl-2-sulfanylbutane; HS-SPME, head space solid-phase micro-extraction; LSD, least significant difference.

3.6 ACKNOWLEDGMENTS

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3.7 LITERATURE CITED

(1) Marais, J., Sauvignon blanc Cultivar Aroma - A Review. *South African Journal of Enology and Viticulture* **1994,** 15, 41-45.
(2) Swiegers, J. H.; Francis, I. L.; Herderich, M. J.; Pretorius, I. S., Meeting consumer expectations through management in vineyard and winery: the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon blanc wine. *Austral. N.Z. Wine Ind. J.* **2006,** 21, 34-42.
(3) Singleton, V. L.; Zaya, J.; Trousdale, E.; Salgues, M., White table wine quality and polyphenol composition as affected by must SO2 content and pomace contact time. *Am. J. Enol. Vitic* **1980,**31, (1), 14-20.
(4) Marais, J., Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *S. Afr. J. Enol. Vitic.* **1998,** 19, (1), 10-16.
(5) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V., Methoxypyrazines in Sauvignon blanc grapes and wines. *Am. J. Enol. Vitic* **1991,** 42, 103-108
(6) Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V., Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic* **1991,** 42, (2), 109-112.
(7) Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C., Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969,** 17, 1322-1327.
(8) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.; Dubourdieu, D., Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-Mercapto-4-methylpentan-2-one. *Flavour and Fragrance Journal* **1995,** 10, 385-392.

(9) Tominaga, T.; Darriet, P.; Dubourdieu, D., Identification of 3-sulfanylhexyl acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor. *Vitis* **1996,** 35, 207-210.
(10) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D., Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour and Fragrance J.* **1998a,** 13, 159-162.
(11) Tominaga, T.; Murat, M.-L.; Dubourdieu, D., Development of a method analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **1998,** 46, 1044-1048.
(12) Dubourdieu, D.; Tominaga, T.; Masneuf, I.; Peyrot des Gahons, C.; Murat, M. L., The role of yeast in grape flavour development during fermentation: The example of Sauvignon blanc. *Am. J. Enol. Vitic* **2006,** 57, (1), 81-88.
(13) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D., *Handbook of Enology*. 2 ed.; John Wiley & Sons Ltd: Chichester, 2006; Vol. 2.
(14) Lund, C. M.; Thompson, M. K.; Benkwitz, F.; Wohler, M. W.; Triggs, C. M.; Gardner, R.; Heymann, H.; Nicolau, L., New Zealand Sauvignon blanc distinct flavor characteristis: Sensory, chemical, and consumer aspects. *Am. J. Enol. Vitic* **2009,** 60, 1-12.
(15) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D., A new type of flavour precursors in Vitis vinifera L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem.* **1998b,** 46, 5215-5219.
(16) Peyrot des Ganchos, C.; Tominaga, T.; Dubourdieu, D., Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **2002,** 50, 4076-4079.
(17) Fedrizzi, B.; Pardon, K. H.; Sefton, M. A.; Elsey, G. M.; Jeffery, D. W., First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan- 2-one, in Sauvignon blanc juice. *J. Agric. Food Chem.* **2009,** 57 (3), 991-995.
(18) Swiegers, J. H.; Capone, D. L.; Pardon, K. H.; Elsey, G. M.; Sefton, M. A.; Francis, I. L.; Pretorius, I. S., Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. *Yeast* **2007,** 24, 561-574.
(19) Roland, A.; Vialaret, J.; Razungles, A.; Rigou, P.; Schneider, R., Evolution of *S*-Cysteinylated and *S*-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. *J. Am. Chem. Soc.* **2010,** 58, 4406-4413.
(20) Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E., *Principles and practice of winemaking*. Chapman & Hall: New York, 1996.
(21) Jocelyn, P. C., Biochemistry of the SH group. The occurance, chemical properties, metabolism and biological function of thiols and disulphides. In Academic Press: London, UK, 1972.
(22) Kotserides, Y.; Ray, J.; Augier, C.; Baumes, R., Quantitative determination of sulfur containing wine odorants at sub-ppb levels. 1. Synthesis of the deuterated analogues. *J. Agric. Food Chem.* **2000,** 48, 5819-5823.
(23) Blanchard, L.; Darriet, P.; Dubourdieu, D., Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic* **2004,** 55, 115-120.
(24) Brajkovich, M.; Tibbits, N.; Peron, G.; Lund, C. M.; Dykes, S. I.; Kilmartin, P. A.; Nicolau, L., Effect of screwcap and cork closures on SO_2 levels and aromas in Sauvignon blanc wine. *J.Agric. Food Chem.* **2005,** 53, (26), 10006-10011.
(25) Lopes, P.; Silva, M. A.; Pons, A.; Tominaga, T.; Lavigne, V.; Saucier, C.; Darriet, P.; Teissedre, P. L.; Dubourdieu, D., Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. *J. Agric. Food Chem.* **2009,** 57, 10261-10270.
(26) Ribéreau-Gayon, P.; Glories, Y.; Dubourdieu, D.; Maujean, A., Traite d'oenologie. Chimie du vin. Stabilisation et traitements. In Dunod Paris, 1998, 2004b.
(27) Cheynier, V.; Trousdale, E.; Singleton, V. L.; Salgeus, M.; Wylde, R., Characterization of 2-S-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. *J. Agric. Food Chem.* **1986,** 34, 217-221.
(28) Darriet, P. *Caracterisation des composes volatils associes a la vigne et au vin.*; Universite Victor Segalen Bordeauxm: 2002; p 97.
(29) Nikolantonaki, M.; Chichuc, I.; Teissedre., P. L.; Darriet, P., Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. *Analytica Chimica Acta* **2010,** 660, 102-109.
(30) Singleton, V. L., Oxygen with phenols and related reactions in must, wines and modelsystems: observations and practical implications. *Am. J. Enol. Vitic* **1987,** 38, 69-77.
(31) Nikolantonaki, M.; Jourdes, M.; Quideau, S.; Teissedre, P.-L.; Darriet, P. In *Characterization and formation kinetic study of phenolic compounds-volatile thiol adducts by chemical and enzymatic oxidation*, Third International Symposium on macromolecules and secondary metabolites of grapevine and wine, Torino, Italy, 2010; Torino, Italy, 2010; p 5.
(32) Singleton, V. L.; Salgues, J.; Zaya, J.; Trousdale, E., Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. *Am. J. Enol. Vitic* **1985,** 36, 50-56.

(33) Cheynier, V., Masson, G., Rigaud, J., Moutounet, M., Estimation of must oxidation during pressing in Champagne. *Am. J. Enol. Vitic* **1993,** 44, (4), 393-399.
(34) Dubernet, M.; Ribereau-Gayon, P., Causes et consequences de la consummation de l'oxygene par les mouts des raisin. *Vitis.* **1974**, 13, 233-244.
(35) Patel, P.; Herbst-Johnstone, M.; Lee, S. A.; Gardner, R. C.; Weaver, R.; Nicolau, L.; Kilmartin, P. A., Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of Sauvignon blanc microferments. *J. Agric. Food Chem.* **2010,** 58, 7280-7288.
(36) Du Toit, W. J.; Lisjak, K.; Stander, M.; Prevoo, D., Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. *J. Agric. Food Chem.* **2007,** 55, (8), 2765-2769.
(37) Peng, Z.; Iland, P. G.; Oberholster, A.; Sefton, M. A.; Waters, E. J., Analysis of pigmented polymers in red wine by reverse phase HPLC. *Australian Journal of Grape and Wine Research* **2002,** 8, 70-75.
(38) Hebditch, K. R.; Nicolau, L.; Brimble, M. A., Synthesis of isotopically labelled thiol volatiles and cysteine conjugates for quantification of Sauvignon blanc wine. *J. Label Compd Radiopharm* **2007,** 50, 237-243.
(39) Parr, W. V.; Green, J. A.; White, K. G.; Sherlock, R. R., The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. *Food Quality and Preference* **2007,** 18, 849-861.
(40) Cheynier, V., Souquet, J.M., Moutounet, M., Glutathione content and glutathione to hydroxycinnamic acid racio in *vitis vinifera* grapes and musts. *Am. J. Enol. Vitic* **1989,** 40,320-324.
(41) Roussis, I. G.; Lambropoulos, I.; Tzimas, P., Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *Am. J. Enol. Vitic* **2007,** 58, (2), 274-278.
(42) Ribéreau-Gayon, P.; Dubourdieu, D.; Doneche, B.; Lonvaud, A., *Handbook of Enology*.2 ed.;John Wiley & Sons Ltd: Chichester, 2006; Vol. 1.
(43) Lavigne, V.; Pons, A.; Dubourdieu, D., Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection changes in concentration in white wines during alcoholic fermentation and aging. *J.Chromatogr.* **2007,** 1139, 130-135.
(44) Du Toit, W. J.; Marais, J.; Pretorius, I. S.; du Toit, M., Oxygen in wine: A review. *S. Afr.J. Enol. Vitic.* **2006,** 27, (1), 76-94.
(45) Maggu, M.; Winz, R.; Kilmartin, P. A.; Trought, M. C. T.; Nicolau, L., Effect of skin contact and pressure on the composition of Sauvignon blanc must. *J. Agric. Food Chem.* **2007,** 55, 10281-10288.
(46) Gürbüz, O.; Göçmen, D.; Dağdelen, F.; Gürsoy, M.; Aydin, S.; Şahin, I.; Büyükuysal, L.; Usta, M., Determination of flavan-3-ols and trans-resveratrol in grapes and wine using HPLC with fluorescence detection. *Food Chem* **2007,** 100, 518-525.
(47) Cheynier, V.; Van Hulst, M. W. J., Oxidation of trans-caftaric acid and 2-S-glutathionyl caftaric acid in model solutions. *J. Agric. Food Chem.* **1988,** 36, 10-15.
(48) Cheynier, V.; Ricardo da Silva, J. M., Oxidation of grape procyanidins in model solution containing trans-caffeoyltartaric acid and polyphenol oxidase. *J.Agric. Food Chem.* **1991,** 39, 1047-1051.
(49) Danilewicz, J. C.; Seccombe, J. T.; Whelan, J., Mechanism of interaction of polyphenols, Oxygen, and Sulphur dioxide in model wine and wine. *Am. J. Enol. Vitic* **2008,** 59, (2), 128-136
(50) Anon *TNO, Volatile compounds in foods. Qualitative and quantitative data*; Nutrition and food research institute: Zeist, The Netherlands, 1996; p 823.
(51) Larue, F.; Lafon-Lafourcade, S.; Ribéreau-Gayon, P., Relationship between the sterol content of yeast cells and their fermentation activity in grape must. *Appl. Environ. Microbiol.* **1980,** 39, 808-811.
(52) Vaimakis, V.; Roussis, I. G., Must oxygenation together with glutathione addition in the oxidation of white wine. *Food Chemistry* **1996,** 57, 419-422.
(53) Subileau, M.; Schneider, R.; Salmon, J. M.; Degryse, E., New insights on 3-mercaptohexanol (3SH) biogenesis in Sauvignon blanc wines: Cys-3SH and (E)-Hexen-2-al are not the major precursor. *J. Agric. Food Chem.* **2008,** 56, 9230-9235.

Chapter 4

Research results

Effect of must oxygenation and sulphur dioxide addition on esters, higher alcohols, fatty acids and terpenes in Sauvignon blanc wine

4. Effect of must oxygenation and sulphur dioxide addition on esters, higher alcohols, fatty acids and terpenes in Sauvignon blanc wine

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ABSTRACT

Sauvignon blanc wines often have complex aromatic profiles with flavours ranging from herbaceous to tropical. Yeast derived aroma compounds can also contribute significantly to the overall flavour. Oxygen and sulphur dioxide additions to the must can influence the yeast performance and possibly the formation of these volatile compounds. However, the effect of different oxygen and sulphur dioxide additions to Sauvignon blanc must and the changes it induces in the corresponding wines has not been investigated in detail. The present study evaluated how different oxygen and sulphur dioxide treatments of Sauvignon blanc must affects the levels of volatile aroma compounds such as acetate esters, ethyl esters, higher alcohols, fatty acids and monoterpenes. No clear trend was found within the aroma groups, although it was evident that the presence of SO_2 resulted in wines with higher concentrations of certain compounds. However, the effect of oxygen was for the most part, not significant

KEYWORDS: Sauvignon blanc; wine aroma; volatile aroma; monotepenes; oxygen; sulphur dioxide

4.1 INTRODUCTION

The aroma of Sauvignon blanc wines are often described as being vegetative, grassy or green pepper caused by the grape derived compound, methoxypyrazines (*1*). Aroma compounds such as the volatile thiols can contribute tropical, passion fruit and guava-like nuances and originate from precursors in the grape, which are released by the yeast during fermentation (*2, 3*). Other than these characteristic and cultivar specific aroma compounds, other yeast-derived compounds can also have a significant impact on the aroma bouquet of a wine. Yeast fermentation of sugar does not exclusively produce ethanol and carbon dioxide, but a wide range of other sensorial important volatile metabolites. The volatile compounds synthesized by wine yeasts include acetate and ethyl esters, higher alcohols and volatile fatty acids (*4-7*).

These compounds appear to be generic to most wine cultivars (*8*). However, strong correlations have been found between grape variety and the main by-products of yeast amino acid metabolism, specifically iso-acids and certain aroma compounds (*8, 9*). Recent studies could not find any significant

differences in ester (except for hexyl acetate and ethyl hexanoate), higher alcohol and fatty acid (except for decanoic and octanoic acids) concentrations between Sauvignon blanc and Chardonnay wines (*10*).

Esters are produced by the yeast during fermentation and their aroma are generally described as being pleasant, fruity and even floral (*5, 12*). Esters are formed due to the condensation of an alcohol and a coenzyme-A-activated acid (acyl-CoA), brought about by the action of alcohol acetyl transferases (*5*). In the same manner, ethyl esters are generated from acyl-CoA and ethanol. Some of the most significant esters in wine has been identified as isoamyl acetate, ethyl hexanoate and 2-phenylethyl acetate (*13*). Higher alcohols often display solvent-like, marzipan and even floral notes and can be anabolically synthesized from intermediates of the sugar metabolism or catabolically synthesized from branch-chain amino acids, through the Ehrlich pathway (*5, 14-17*). Fatty acids are essential constituents of the plasma membrane and are precursors of more complex molecules. Fatty acids can be synthesized by the repetitive condensation of acetyl-CoA by the action of the fatty acid synthetase complex (*5*). Fatty acids can contribute to the aroma of a wine at low concentrations, but at higher concentrations cause unpleasant rancid and cheese like odours (*18*).

Different factors can affect the formation of these yeast derived compounds. Juice clarification, yeast strain and fermentation temperature are all variables that can greatly influence the final concentration of yeast-derived aroma compounds in wine (*8, 9, 19-23*). The effect of wine storage or wine exposure to oxygen on volatile aroma compounds have been investigated and it is known to cause a general decrease in positive aroma compounds with an increase in unwanted flavours (*14, 24-27*). However, the mechanism that causes esters to disappear during wine storage is unclear. Volatile losses in wine might be due to oxidation or other chemical reactions. It has been proposed that ester concentrations can change due to hydrolysis and estrification (*28*) or oxidation by hydroxyl-radical oxidation related processes (*29, 30*).

The aeration of grape must prior to fermentation occurs often as a result of its transfers in industrial practices. Oxygenation at the onset or during alcoholic fermentation can have an enhancing effect on the yeast as it modifies the yeast metabolism by increasing sterol biosynthesis and favouring cellular growth (*31*). This leads to improved ethanol tolerance, fermentative capability and viability (*32*). There seems to be a close relationship between fermentation rate/yeast growth and ester production (*12, 33*). On the other hand, aeration of grape must can cause oxidation of polyphenols due to the polyphenoloxidase enzyme (PPO) (*34-36*). Polyphenols such as *trans*-caftaric acid serves as a substrate for PPO, forming *o*-quinones. These *o*-quinones can partake in further oxidation reactions and form brown pigments, or associate with glutathione (GSH) to form the grape reaction product (GRP) (*35, 37*).

There seem to be contradicting results on the effect of oxygen addition, prior to fermentation, on the formation of volatile aroma compounds. Valero *et al*., 2002 investigated the effect of oxygen addition prior to fermentation on ester and higher alcohol production and found an increase in higher alcohols and certain esters concentrations in wines made from must with prior oxygenation (*38*). This increase

can be ascribed to the increased growth of the yeast. It is unclear whether SO_2 was used in this study. Mauricio *et al*., 1997 also found an increased production of isoamyl acetate, phenethyl acetate, isoamyl alcohol and 2-phenyl ethanol, in semi-aerobic fermentation conditions when compared to anaerobic conditions (*39*). However, the semi-aerobic conditions in this study did not include agitation of juice with air; it only allowed the fermenting juice to be in contact with air. In contrast, the addition of oxygen to the must have also been found to increase ethyl ester concentrations, but decreased the concentrations of some higher alcohols and acetate esters (*40*).

Medium chain fatty acids (MCFA) such as hexanoic, octanoic and decanoic acids accumulate in the yeast with anaerobic fermentation conditions and can be secreted in the wine, which could result in stuck or sluggish fermentations due to higher concentrations of these compounds present (*41*).

Previous studies reported varying results when determining the concentration of volatile aroma compounds of wines made with different SO_2 additions to the must. Marais, 1998 and Garde-Cerdán *et al*., 2007 could not find consistent differences between the ester content in wines made from juice with and without SO_2 addition prior to fermentation. However, in other studies, higher acetate and ethyl ester concentrations was found in wines made with SO_2 when compared to wines made from juice with no SO_2 additions (*22, 42*) and hyperoxidised juice (*43*). The formation of the alcohols, isoamyl alcohol and 2-phenyl ethanol, was significantly greater in fermentations conducted with SO_2 than in a those with no SO_2 addition. However, the presence or absence of SO_2 had no significant effect on the formation of MCFAs (*44*).

Monoterpenes and monoterpene alcohols are known for their floral, fruity, citrus and perfume odours usually expressed by geraniol, linalool, nerol and α-terpineol (*45*). Terpenes are not considered to be a character- impacting compound for Sauvignon blanc as they are usually expressed as an impact odorant for the muscat family of grapes (*46*). Even though terpene concentrations in wine such as Sauvignon blanc, are generally below the perception threshold, the olfactory impact of terpene compounds is often synergistic and can influence the overall complexity of a wine (*46*). A considerable proportion of these compounds are in bound form in the juice, known as aglycones. The hydrolysis of the *O*-glycosyl bond during fermentation by means of yeast β-glycosidase, will release the terpenoid compound, rendering it aromatic, although some aglycons can become aromatic by chemical rearrangement (*47, 48*). Fermentation conditions that stimulate the glycolytic flux, such as high assimilable nitrogen content and aerobic fermentation conditions often result in higher concentrations of monoterpenes in wine. Sulphur dioxide additions prior to skin contact did not have any significant effect on the total terpene content of the settled juices when compared to juices with no SO_2 additions (*49*). Oxidation of Muscat wines can cause a remarkable loss of aroma and an increase in terpenes were found in wines that were made in a CO_2 enriched atmosphere (*50*). During oxidation, most monoterpene alcohols are replaced by terpene oxides, which have a higher perception threshold (*51*). During maturation, the terpenes can undergo transformation and the total terpene content will decrease (*52, 53*).

These yeast derived aroma compounds could all be significant contributors to the overall bouquet of Sauvignon blanc wines and the manipulation of the formation of these compounds could be an important tool in winemaking. Oxygen management has enjoyed great attention in modern wineries although, from literature, it seems that the effect of oxygen and SO_2 additions prior to fermentation in different musts is not uniform and may depend on several factors. The aim of this study was to investigate the effect of different oxygen and SO_2 additions to Sauvignon blanc must on the rate of fermentation, as well as ester, alcohol, fatty acid and monoterpene formation in the corresponding wines.

4.2 MATERIALS AND METHODS

4.2.1 Juice and Winemaking Treatments

The juice treatments and winemaking techniques are described in section 3.2.1.

4.2.2 Sampling procedure

Wine samples were drawn just before bottling and the sampling procedure has been described earlier in section 3.2.2.

4.2.3 Volatile Aroma analyses

Chemicals, standards, and model wine matrix has been described (*10,11*). Five millilitres of wine with internal standard, 4-methyl-2-pentanol (100 µL of 0.5 mg/L solution in 12% ethanol-water mixture), was extracted with 1 mL of diethyl ether by sonicating the ether/wine mixture for 5 minutes. The wine/ether mixture was then centrifuged at 3600g for 3 minutes. The ether layer was removed and dried on Na_2SO_4. Each extract was injected into the GC-FID in duplicate. Validation of the method, in terms of selectivity, linearity, limits of detection, limits of quantification, recovery, robustness, and repeatability, has been described (*53*). A J&W DB-FFAP capillary GC column (Agilent, Little Falls, Wilmington, DE) with dimensions 60 m length × 0.32 mm i.d. × 0.5 µm f.t. and a Hewlett Packard 6890 Plus GC (Little Falls, DE) equipped with a split/splitless injector and FID detector were used. The initial oven temperature was 33 ^{o}C for 17 min, after which the temperature was increased by 12 ^{o}C/min to 240 ^{o}C, at which it was held for 5 min. Three microlitres of the diethyl ether extract was injected at 200 ^{o}C. The split ratio was 15:1, and the split flow rate was 49.5 mL/min. The column flow rate was 3.3 mL/min using hydrogen as a carrier gas. The detector temperature was 250 ^{o}C, with a column flow of 6 mL/min, cleaned the column of high boiling contaminants. Quantification was performed by comparing the ratio of the peak area and internal standard peak area with calibration graphs constructed using pure standards (*10,11*).

4.2.4 Monoterpenes analyses

The solid phase extraction was performed in a Visiprep SPE vacuum manifold 20-port model from Supelco, in which there are 20 positions available for performing the SPE simultaneously. Cartridges (Strata SDB-L, Phenomenex, Torrance, CA, USA) were conditioned by rinsing with 4 ml dichloromethane, 4 ml methanol and finally 4 ml of wine simulant (12% ethanol-water mixture). Internal standard, 50 µl of 2,6-dimethyl-6-hepten-2-ol (25 mg/L made up in ethanol) was added to 50 ml of wine and mixed briefly. The wine was then rinsed through the cartridge by vacuum suction (-0.5 kPa). Clean-up was obtained by flushing the cartridge with 4 ml Milli-Q-Water®. The cartridge was then dried under vacuum (-10 kPa) for 15 minutes. Finally, terpenoids were then eluted from the solid phase using 2 ml dichloromethane. The dichloromethane elute was then dried on sodium sulphate crystals and injected into the GC-FID. Each extract was injected into the GC-FID in duplicate. Separation and quantification were performed on a Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph equipped with a 60 m × 0.32 × 0.5 mm fused DB-FFAP capillary column (J&W Scientific, Folsom, CA), and flame ionization detector (FID). Separation conditions were as follows: injector temperature 200 ^{o}C ; GC column temperature 40 ^{o}C (12 min) at 12 ^{o}C min^{-1} to 190 ^{o}C, followed by a temperature ramp of 15 ^{o}C min^{-1} to a final temperature of 250 ^{o}C (2 min); carrier gas Helium at 40 kPa. Each compound was quantified by comparison with a calibration curve constructed using pure standards. The relative peak area and internal standard peak area were then compared to the calibration curve in order to quantify each compound (*54*).

4.2.5 Data Analyses

All analyses were done using Statistica 9. One-way ANOVA's were conducted with the 4 combinations of SO_2/O_2 levels treated as 4 different treatments (Table 1). These results were in some cases supported by 2-way ANOVA's using SO_2 and O_2 as separate factors (Table 2). Fisher least significant difference (LSD) corrections were used for post-hoc analyses. Significant differences were judged on a 5% significance level ($p<0.05$). For investigating the effect of treatments on the fermentation process, growth curves of the form percentage weight = Lowest value % + (100% - Lowest value %)/(1+10^((EC50-v4)*Slope)) were fitted on the data over time. The EC50 parameter indicates at what time the percentage weight decreased by half of the total decrease.

4.3 RESULTS AND DISCUSSION

4.3.1 Fermentation performance

Although all treatments completed alcoholic fermentation at the same time, differences between the fermentation rates in the growth phase were observed between treatments in cellar 2. A growth curve was constructed (Figure 1) and the EC50 (number of days required to reach the half way mark) was

calculated (not shown). The EC50 value was significantly different for each treatment with treatment C progressing the fastest and treatments A, D and B following in descending order. The treatments with no SO_2 additions (treatments C and A) progressed the fastest as there was no antimicrobial action in the form of added SO_2 to delay the yeast growth. Musts which received oxygen prior to fermentation also fermented at a faster rate when compared to treatments with no O_2 addition as oxygen would enhance the cellular growth and performance of the yeast cells. However, cellar 1 had no significant difference between the fermentation rates for the different treatments (results not shown).

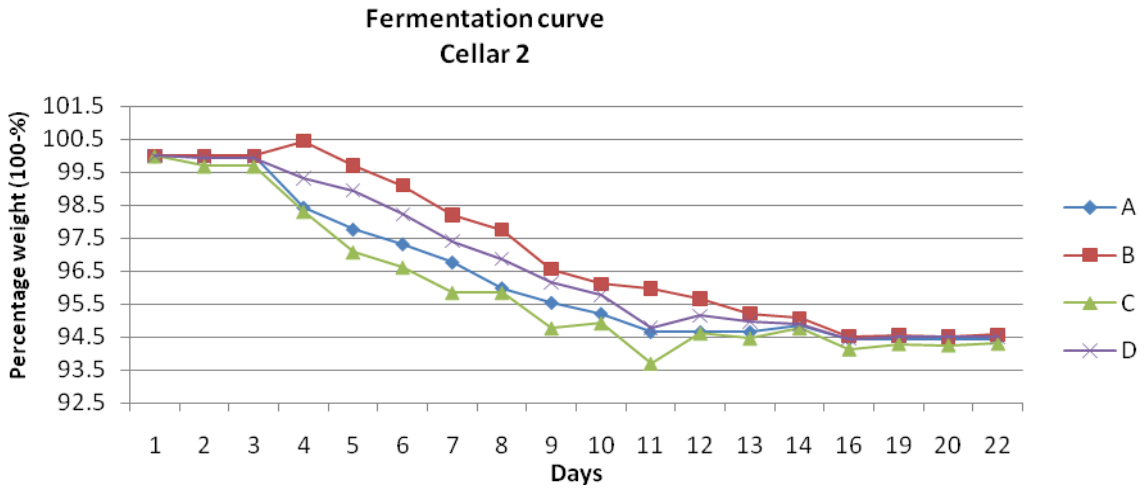

Figure 1. Fermentation curve of juice undergoing different SO_2 and O_2 treatments from cellar 2. Mass of fermentation vessels presented as percentage weight. A ($-SO_2/-O_2$); B ($+SO_2/-O_2$); C ($-SO_2/+O_2$); D ($+O_2/+SO_2$).

4.3.2 Volatile compounds

Table 1 lists the higher alcohol, ester, fatty acid and monoterpene contents of the wines after fermentation. Table 2 shows the significance of the effect of oxygen addition, SO_2 addition and the interaction between these treatments on a 5% confidence interval. It is clear that SO_2 addition significantly influenced the majority (36% and 42% for cellar 1 and 2 respectively) of the volatile compounds in the wine, which will be discussed in more detail.

The acetate esters, isoamyl acetate and 2-phenylethyl acetate, for cellar 1 were found to be mostly higher in the treatments with antioxidant protection (treatments B and D), although these differences were not always significant (Table 1). This corresponds with the results found by Daudt *et al.,* 1973 and Moio *et al*., 2004. It would seem as if the combined effect of oxygen and SO_2 did have an influence on the concentrations of isoamyl acetate and 2-phenylethyl acetate from cellar 2, however the effect of O_2 alone was not significant for both of the cellars for these compounds (Table 2).

The ethyl esters ethyl hexanoate, ethyl octanoate and ethyl decanoate, and the MCFA, hexanoic acid, octanoic acid and decanoic acid also followed the same tendency as the acetate esters, mostly being produced in higher amounts in the treatments with added SO_2. The differences between the treatments

became less evident with the increase in molecular weight of the compounds. These results are contradictory with previous studies that report an increase in specifically MCFA in anaerobic fermentation conditions (*41*). The effect of oxygen addition also significantly influenced the levels of ethyl hexanoate and ethyl octanoate in the wines from cellar 1 as the addition of oxygen (treatment C and D) resulted in relatively higher concentrations when compared to treatments with no oxygen addition. This was also found in studies done by Bertrand *et al.,* 1984. Except for hexyl acetate (cellar 1) and ethyl butyrate (cellar 2), the effect of O_2 addition was not significant for the rest of the esters for the conditions of this study. The addition of sulphur dioxide thus seemed to have been the main influencing factor (Table 2). The contents of other esters and fatty acids acids (such as ethyl acetate, ethyl lactate, diethyl succinate, propionic acid, isobutyric acid, butyric acid, iso-valeric acid and valeric acid) exhibited no clear trends under the different treatments. Acetic acid did however show an increased concentration (although not significant) in treatments where no SO_2 was added. This is probably due to the survival of acetic acid bacteria or other non-*Saccharomyces* yeast, which can produce acetic acid, in the treatments with no SO_2 additions (treatments A and C) (*56*).

The higher alcohols, isoamyl alcohol and 2-phenyl ethanol was less affected by the treatments and did not show significant differences between the treatments. However, the level of 2-phenyl ethanol from cellar 1 was influenced by both the effect of oxygen addition and SO_2 addition. Sulphur dioxide had a significant protective effect and the addition of O_2 caused the levels of 2-phenyl ethanol to decrease. This corresponds with results by Garde-Cerdán *et al.,* 2007 and Bertrand *et al.,* 1984. Other alcohols did not show clear trends, except for hexanol which occurred at higher concentrations in wines from treatment C from cellar 1 (Table 1) which have also been found in literature (*57*).

It would seem as if SO_2 had a protective effect on α-terpineol in wine from cellar 1, resulting in higher concentrations. In the case of farnesol, O_2 additions have caused an increase in the occurrence of this compound in the wine from only cellar 2.

It is clear that the biggest effect on the volatile aroma composition of the wines was due to the addition of SO_2. Moio *et al*., 2004 also found higher levels of these compounds in treatments with higher antioxidant activity due to SO_2 additions. They ascribed the increased concentrations to the absence of oxygen during fermentation (although oxygen levels before yeast inoculation was not reported). In this study, the effect of SO_2 addition to the must was significant for most of the compounds, despite the presence of oxygen in treatment D at the onset of fermentation. The proposed explanation could be the inhibition of individual enzymes responsible for the formation of certain compounds, while other enzymes are not sensitive to SO_2 additions (*22*). Another possibility could be the suppression of natural flora present in the must, by the SO_2. Natural microbes present on the grapes at the time of harvest, could provide competition for the inoculated yeast and could cause the formation of compounds other than the preferred volatiles. Commercial yeast strains are more resistant to SO_2 additions leading to a “cleaner” fermentation in the treatments with added SO_2 (*58*).

It would seem as if there was little correlation between fermentation rate and the production of volatile compounds by the yeast. To the contrary, the two treatments with the lowest fermentation rates, treatment B and D, produced the highest amount of volatiles in general. All the above mentioned compounds (except for α-terpineol) were found in concentrations higher than their perception thresholds (Table 1) and could possibly have a significant impact on the aroma perceived. It therefore seems that the winemaker can manipulate the aromatic composition of Sauvignon blanc by adding different concentrations of O_2 and SO_2 to the must. However, more research is required on this subject.

Table 1. Concentration (mg/L)* of volatile compounds found in wines from cellar 1 and cellar 2

	Cellar 1				Cellar 2			
	No oxygen addition		Oxygen addition		No oxygen addition		Oxygen addition	
	No SO_2 addition	SO_2 addition	No SO_2 addition	SO_2 addition	No SO_2 addition	SO_2 addition	No SO_2 addition	SO_2 addition
compound	A	B	C	D	A	B	C	D
higher alcohols								
isoamyl alcohol[ξ]	148.7(1.56)[a]	150.4(9.79)[a]	147.9(7.71)[a]	161.5(14.44)[a]	181.2(20.37)[a]	169.8(1.31)[a]	169.4(5.64)[a]	162.6(17.43)[a]
2-phenyl ethanol[ξ]	14.3(0.23)[a]	15.2(1.37)[a]	12.7(0.62)[b]	14.3(0.82)[a]	13.2(1.34)[a]	13.9(0.32)[a]	12.2(0.62)[a]	13.1(1.35)[a]
hexanol	0.8(0.05)[a]	0.8(0.02)[a]	1.1(0.04)[b]	0.9(0.04)[c]	1.3(0.14)[a]	1.3(0.05)[a]	1.4(0.09)[a]	1.3(0.01)[a]
methanol	84.6(23.74)[b]	41.9(6.94)[a]	45.2(9.21)[a]	41.4(1.35)[a]	83.2(9.76)[a]	70.4(0.87)[a]	75.2(5.81)[a]	70.5(10.37)[a]
propanol	38.8(0.01)[a]	29.5(0.02)[b]	39.9(0.00)[a]	34.4(0.03)[ab]	42.5(3.47)[a]	25.1(1.28)[b]	39.2(2.48)[a]	27.2(6.08)[b]
butanol	0.8(0.01)[a]	0.7(0.03)[a]	0.8(0.09)[a]	0.7(0.03)[a]	1.2(0.10)[a]	1.0(0.07)[b]	1.0(0.05)[ab]	1.0(0.12)[ab]
isobutanol	23.2(1.30)[ab]	22.9(0.67)[ab]	22.4(1.21)[a]	24.8(1.83)[b]	25.7(2.91)a	20.5(0.83)[bc]	24.8(2.78)[ab]	20.4(2.77)[c]
total alcohols	310.2(25.83)[a]	258.5(16.25)[b]	269.8(17.97)[b]	278.1(19.22)[ab]	348.6(29.76)[a]	304.8(4.94)[a]	323.2(15.43)[a]	309.3(34.52)[a]
esters								
isoamyl acetate[ξ]	8.2(0.58)[ab]	8.9(0.16)[a]	7.0(0.89)[b]	9.2(1.53)[a]	14.6(1.47)[a]	13.1(0.10)[a]	13.1(0.43)[a]	15.1(2.10)[a]
2-phenylethyl acetate[ξ]	0.85(0.01)[ab]	1.0(0.18)[c]	0.8(0.08)[a]	1.0(0.05)[bc]	1.0(0.07)[ab]	0.8(0.07)[ac]	0.8(0.13)[c]	1.0(0.09)[b]
ethyl hexanoate[ξ]	1.7(0.07)[a]	1.9(0.10)[a]	1.9(0.08)[ab]	2.1(0.10)[c]	1.7(0.17)[a]	2.0(0.10)[b]	1.6(0.11)[a]	2.1(0.12)[b]
ethyl octanoate[ξ]	0.9(0.03)[a]	1.1(0.11)[bc]	1.0(0.12)[ab]	1.3(0.17)[c]	1.6(0.47)[a]	2.3(0.10)[b]	1.5(0.16)[a]	2.6(0.31)[b]
ethyl decanoate[ξ]	0.6(0.10)[a]	0.8(0.08)[a]	0.6(0.14)[b]	0.8(0.11)[a]	0.8(0.06)[a]	0.8(0.08)[a]	0.7(0.04)[a]	1.0(0.14)[b]
ethyl acetate[ξ]	116.3(9.59)[a]	114.3(11.23)[a]	100.3(8.32)[a]	110.1(9.08)[a]	165.6(15.99)[b]	107.2(6.34)[a]	131.1(20.79)[a]	130.1(20.56)[a]
ethyl butyrate[ξ]	0.8(0.01)[ab]	0.8(0.02)[ab]	0.8(0.00)[a]	0.8(0.03)[b]	0.8(0.06)[a]	0.8(0.05)[a]	0.7(0.04)[b]	0.8(0.02)[ab]
hexyl acetate	0.8(0.81)[a]	0.8(0.84)[ab]	0.9(0.88)[b]	0.9(0.86)[b]	1.1(0.07)[a]	1.1(0.06)[ab]	1.1(0.11)[a]	1.2(0.02)[b]
ethyl lact ate	3.1(0.59)[a]	3.8(0.25)[ab]	3.6(0.28)[ab]	4.0(0.55)[b]	3.9(0.13)[a]	3.6(0.11)[a]	3.6(0.07)[a]	3.6(0.40)[a]
diethyl succinate	0.6(0.02)[a]	0.6(0.01)[a]	0.6(0.05)[a]	0.7(0.03)[b]	0.8(0.07)[a]	0.8(0.03)[a]	0.8(0.10)[a]	0.8(0.06)[a]
total esters	129.2(3.29)[ab]	128.3(2.59)[ab]	116.7(8.83)[a]	130.8(11.09)[b]	191.6(18.17)[a]	131.3(8.54)[b]	153.6(23.64)[bc]	164.3(20.16)[ac]
fatty acids								
acetic acid	1008.8(154.07)[a]	771.7(24.06)[b]	863.7(4.72)[ab]	801.2(78.64)[b]	987.1(237.37)[a]	708.1(79.81)[a]	1018.5(89.74)[a]	792.2(66.55)[a]
hexanoic acid[ξ]	4.9(0.42)[a]	5.2(0.20)[ab]	5.0(0.19)[ab]	5.4(0.18)[b]	4.4(0.53)[ab]	5.1(0.38)[ac]	4.0(0.47)[b]	5.3(0.45)[c]
octanoic acid[ξ]	7.2(0.44)[a]	7.9(0.08)[b]	7.6(0.62)[ab]	7.6(0.31)[ab]	5.1(0.43)[a]	6.7(0.74)[b]	4.9(0.54)[a]	6.3(0.63)[b]
decanoic acid[ξ]	2.7(0.15)[a]	2.9(0.06)[a]	2.8(0.35)[a]	2.8(0.12)[a]	1.3(0.04)[a]	1.4(0.17)[ab]	1.3(0.09)[ab]	1.5(0.08)[b]
propionic acid	1.5(0.56)[a]	1.3(0.08)[a]	1.5(0.10)[a]	1.5(0.03)[a]	1.6(0.17)[ab]	1.3(0.07)[a]	1.7(0.11)[b]	1.3(0.30)[a]
isobutyric acid	3.1(0.53)[a]	1.6(0.30)[a]	1.7(0.35)[a]	1.7(0.09)[a]	1.7(0.16)[a]	1.2(0.11)[a]	1.7(0.62)[a]	1.8(0.41)[a]
butyric acid[ξ]	1.0(0.03)[a]	1.1(0.06)[ab]	1.1(0.07)[a]	1.2(0.05)[b]	1.2(0.15)[ab]	1.2(0.12)[a]	1.1(0.07)[b]	1.2(0.04)[a]
iso-valeric acid[ξ]	0.1(0.00)[a]	0.1(0.00)[a]	0.1(0.04)[b]	0.1(0.00)[a]	0.1(0.00)[a]	0.1(0.04)[a]	0.1(0.00)[a]	0.1(0.00)[a]
valeric acid	0.3(0.03)[a]	0.3(0.02)[a]	0.3(0.06)[a]	0.3(0.01)[a]	0.3(0.03)[a]	0.3(0.01)[a]	0.3(0.01)[a]	0.3(0.07)[a]
total acids	1030.0(155.36)[a]	792.2(23.97)[b]	962.8(137.81)[ab]	821.8(79.05)[b]	1002.8(238.12)[a]	668.2(112.84)[b]	1033.5(89.31)[a]	802.1(54.06)[ab]
monoterpenes								
linalooloxide	8.8(0.98)[a]	8.9(0.76)[a]	8.4(0.24)[a]	8.4(1.08)[a]	9.1(0.37)[a]	9.2(1.24)[a]	9.2(1.23)[a]	11.5(0.78)[b]
α-terpeneol	46.4(9.62)[a]	56.3(3.24)[ab]	46.8(5.29)[ab]	56.8(4.11)[b]	86.3(6.15)[a]	72.8(3.43)[b]	74.0(4.16)[b]	88.5(5.69)[a]
β-farnesol[ξ]	134.7(15.60)[a]	143.4(14.79)[a]	148.6(1.81)[a]	140.3(14.73)[a]	137.7(12.39)[a]	127.4(12.75)[a]	182.0(23.12)[b]	169.3(18.61)[b]
total monoterpenes	183.2(27.63)[a]	209.0(19.87)[a]	203.8(4.22)[a]	205.4(14.22)[a]	233.1(11.74)[ab]	209.3(16.49)[a]	265.1(23.49)[bc]	273.9(23.52)[c]

*Values are means(sd) of triplicate analysis; different letters within a cellar and within a row denote significant differences at $p<0.05$.

[ξ]Compounds occuring in the wine in concentrations higher than their perception thresholds.

Table 2. Probability values (p-value) for the effect of oxygen addition, sulphur dioxide addition separately and the interaction between these two factors (combined effect) on volatile aroma compounds in wines. Values in red show factors significantly affecting the wine volatiles at a 5% significance level.

	Cellar 1			Cellar 2		
compound	Oxygen	Sulphur Dioxide	Oxygen*Sulphur Dioxide	Oxygen	Sulphur Dioxide	Oxygen*Sulphur Dioxide
alcohols						
isoamyl alcohol	0.201	0.307	0.179	0.390	0.420	0.515
2-phenyl ethanol	0.034	0.018	0.092	0.237	0.175	0.754
hexanol	0.000	0.000	0.006	0.304	0.368	0.236
methanol	0.015	0.011	0.026	0.538	0.325	0.305
propanol	0.199	0.007	0.408	0.998	0.000	0.174
butanol	0.959	0.376	0.952	0.849	0.120	0.035
isobutanol	0.318	0.142	0.150	0.918	0.013	0.442
total alcohols	0.377	0.085	0.025	0.476	0.067	0.312
esters						
isoamyl acetate	0.397	0.034	0.241	0.906	0.757	0.017
2-phenylethyl acetate	0.511	0.001	0.177	0.989	0.398	0.003
ethyl hexanoate	0.012	0.007	0.376	0.994	0.000	0.199
ethyl octanoate	0.021	0.040	0.345	0.771	0.001	0.265
ethyl decanoate	0.582	0.056	0.170	0.153	0.011	0.314
ethyl acetate	0.216	0.371	0.128	0.758	0.013	0.005
ethyl butyrate	0.796	0.070	0.166	0.039	0.295	0.532
hexyl acetate	0.002	0.656	0.215	0.224	0.016	0.429
ethyl lact ate	0.848	0.044	0.684	0.522	0.258	0.470
diethyl succinate	0.149	0.102	0.238	0.081	0.941	0.556
total esters	0.286	0.169	0.123	0.807	0.033	0.005
acids						
acetic acid	0.765	0.012	0.445	0.328	0.005	0.538
hexanoic acid	0.714	0.027	0.357	0.517	0.003	0.261
octanoic acid	0.845	0.205	0.376	0.337	0.001	0.792
decanoic acid	0.997	0.283	0.347	0.222	0.025	0.863
propionic acid	0.326	0.272	0.867	0.325	0.011	0.873
isobutyric acid	0.017	0.002	0.013	0.250	0.316	0.215
butyric acid	0.050	0.015	0.981	0.307	0.046	0.373
iso-valeric acid	0.062	0.062	0.062	0.260	0.260	0.260
valeric acid	0.920	0.066	0.538	0.838	0.235	0.365
total acids	0.764	0.012	0.446	0.329	0.005	0.534
monoterpenes						
linalooloxide	0.958	0.702	0.700	0.041	0.041	0.054
α-terpeneol	0.748	0.017	0.852	0.328	0.595	0.000
β-farnesol	0.374	0.731	0.192	0.001	0.311	0.980
total monoterpenes	0.422	0.210	0.264	0.001	0.488	0.149

4.4 CONCLUSIONS

The formation of volatiles during fermentation is critical for the final aromatic bouquet of a wine. Although it is not always character impact compounds of Sauvignon blanc wines, volatiles investigated in this study could contribute significantly to the aromatic profile of the wine. These compounds differed in their reaction to pre-fermentative O_2 and SO_2 treatments due to the complex and unique metabolic formation of each aromatic group. In the conditions of this study no clear tendencies could be observed in terms of the effect of O_2 and SO_2 additions on certain esters, alcohols, fatty acids and terpenes. However, it was evident that the addition of SO_2, had a significant effect on the formation of some of these important compounds. However, these results are preliminary and this aspect needs to be investigated in more detail in future.

4.5 ABBREVIATIONS USED

Acyl-CoA, coenzyme-A-activated acid; MCFA, medium chain fatty acids; PPO, polyphenoloxidase enzyme; GSH, glutathione; GRP, grape reaction product; FID, flame ionization detection; GC, gas chromatography; GC-FID, gas chromatography with flame ionization detection; SPE, solid phase extraction

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4.7 LITERAURE CITED

(1) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V., Methoxypyrazines in Sauvignon blanc grapes and wines. *Am. J. Enol. Vitic* **1991,** 42, 103-108

(2) Dubourdieu, D.; Tominaga, T.; Masneuf, I.; Peyrot des Gachons, C.; Murat, M. L., The role of yeast in grape flavour development during fermentation: The example of Sauvignon blanc. In *ASEV 50th Annual Meeting*, Seattle, USA (American Society of Enology and Viticulture, 2000; p 37.

(3) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.; Dubourdieu, D., Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-Mercapto-4-methylpentan-2-one. *Flavour and Fragrance Journal* **1995,** 10, 385-392.

(4) Delfini, C.; Cocito, C.; Bonino, M.; Schellino, R.; Gaia, P.; Baiocchi, C., Definitive evidence for the actual contribution of yeast in the transformation of neutral precursors of grape aromas. *J. Agric. Food Chem.* **2001,** 49, 5397-5408.

(5) Lambrechts, M. G.; Pretorius, I. S., Yeast and its importance to wine aroma - A review. *S. Afr. J. Enol. Vitic.* **2000,** 21, (Special Issue), 97-129.

(6) Stashenko, H.; Macku, C.; Shibamato, T., Monitoring volatile chemicals formed from must during fermentation. *J. Agric. Food Chem.* **1992,** 40, 2257-2259.

(7) Schreier, P., Flavour composition of wines: a review. *CRC Crit. Rev. Food Sci. Nutr.* **1979,** 12, 59-111.
(8) Ferreira, V.; Lopéz, R.; Cacho, J. F., Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agr* **2000,** 80, 1659-1667.
(9) Lopéz, R.; Ferreira, V.; Hernandez, P.; Cacho, J. F., Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *J. Sci. Food Agr* **1999,** 79, 1461-1467.
(10) Louw, L.; Roux, K.; Tredoux, A.; Tomic, O.; Naes, T.; Nieuwoudt, H. H.; Van Rensburg, P., Characterization of selected South African young cultivar wines using FTMIR Spectroscopy, Gas Chromatography, and Multivariate Data Analysis. *J. Agric. Food Chem.* **2009,** 57, 2623-2632.
(11) Louw, L., 2007. Chemical characterization of South African Young Wines. Thesis, University of Stellenbosch.
(12) Houtman, A. C.; Marais, J.; Du Plessis, C. S., The possibilities of applying present-day knowledge of wine aroma components: Influence of several juice factors on fermentation rate and ester production during fermentation. *S. Afr. J. Enol. Vitic.* **1980,** 1, (1), 27-33.
(13) Thurston, P. A.; Taylor, R.; Ahvenainen, J., Effects of linoleic acid supplements on the synthesis by yeast of lipids and acetate esters. *Journal of the Institute of Brewing* **1981,** 87, 92-95.
(14) Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E., *Principles and practice of winemaking*. Chapman & Hall: New York, 1996.
(15) Dickinson, J. R.; Lanterman, M. M.; Danner, D. J.; Paerson, B. M.; Sanz, P.; Harrison, S. J.; Hewlins, J. E., A 13C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1997,** 272, (43), 26871-26878.
(16) Dickinson, J. R.; Salgado, L. E.; Hewlins, J. E., The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2003,** 278, (10), 8028-8034.
(17) Nykänen, L., Formation and occurance of flavor compounds in wine and distilled alsoholic beverages. *Am. J. Enol. Vitic* **1986,** 37, (1), 84-96.
(18) Francis, I. L.; Newton, J. L., Determining wine aroma from compositional data. In *Advances in wine science*, Blair, R. J., Francis, M.E., Pretorius, I.S., Ed. The Australian Wine Research Instutute: Glen Osmond, Australia, 2005; pp 201-212.
(19) Aragon, P.; Atienza, J.; Climent, M. D., Influence of clarification, yeast type, and fermentation temperature on the organic acid and higher alcohols of Malvasia and Muscatel wines. *Am. J. Enol. Vitic* **1998,** 49, (2), 211-219.
(20) Guth, H., Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997,** 45, 3027-3032.
(21) Torija, M. J.; Beltran, G.; Novo, M.; Poblet, M.; Guillamón, J. M.; Mas, A.; Rozès, N., Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. *Int. J. Food Microbiol* **2003,** 85, 127-136.
(22) Daudt, C. E.; Ough, C. S., Variations in some volatile acetate esters formed during grape juice fermentation temperature, SO_2, Yeast strain, and grape variety. *Am. J. Enol. Vitic* **1973,** 24, (3), 130-135.
(23) Molina, A. M.; Swiegers, J. H.; Varela, C.; Pretorius, I. S.; Agosin, E., Influence of wine fermentation temperature on the synthesis of yeast-derived aroma compounds. *Appl. Microbiol. Biotechnol.* **2007,** 77, 675-687.
(24) Escudero, A.; Asencio, E.; Cacho, J.; Ferreira, V., Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem* **2002,** 77, 325-331.
(25) Silva Ferreira, A. C.; Hogg, T.; De Pinho, P. G., Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003,** 51.
(26) Simpson, R. F., Aroma and compositional changes in wine with oxidation, storage and ageing. *Vitis* **1978,** 17, (274-287).
(27) Marais, J.; Van Wyk, C. J.; Rapp, A., Effect of storage time, temperature and region on the levels of 1,1,6-trimethyl-1,2-dihydronaphthalene and other volatiles, and on quality of Weisser Riesling. *S. Afr. J. Enol. Vitic.* **1992,** 13, 33-44.
(28) Ramey, D. D.; Ough, C. S., Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* **1980,** 28, 928-934.
(29) Escudero, A.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V., Clues about the role of methional as character impact odorant of some oxidized wines. *J. Agric. Food Chem.* **2000,** 48, 4268-4272.
(30) Litchev, V., Influence of oxidation processes on the development of the taste and flavor of wine distillates. *Am. J. Enol. Vitic* **1989,** 40, 31-35.
(31) Larue, F.; Lafon-Lafourcade, S.; Ribéreau-Gayon, P., Relationship between the sterol content of yeast cells and their fermentation activity in grape must. *Appl. Environ. Microbiol.* **1980,** 39, 808-811.
(32) Valero, E.; Millan, C.; Ortega, J. M., Influence of oxygen addition during growth phase on the biosynthesis of lipids in Saccharomyces cerevisiae (M330-9) in enological fermentations. *J. Biosc. Bioeng.* **2001,** 92, 33-38.

(33) Nordström, K. In *Possible control of volatile ester formation in brewing*, Proc. Europ. Brew. Conv., Stockholm, 1965; Stockholm, 1965; pp 195-208.
(34) Singleton, V. L.; Salgues, J.; Zaya, J.; Trousdale, E., Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. *Am. J. Enol. Vitic* **1985,** 36, 50-56.
(35) Singleton, V. L., Oxygen with phenols and related reactions in must, wines and model systems: observations and practical implications. *Am. J. Enol. Vitic* **1987,** 38, 69-77.
(36) Cheynier, V.; Ricardo da Silva, J. M., Oxidation of grape procyanidins in model solution containing trans-caffeoyltartaric acid and polyphenol oxidase. *J.Agric. Food Chem.* **1991,** 39, 1047-1051.
(37) Cheynier, V.; Van Hulst, M. W. J., Oxidation of trans-caftaric acid and 2-S-glutathionyl caftaric acid in model solutions. *J. Agric. Food Chem.* **1988,** 36, 10-15.
(38) Valero, E.; Millan, C.; Ortega, J. M., Higher alcohols and esters production by *Saccharomyces cerevisiae*. Influence of the initial oxygenation of the grape must. *Food Chem* **2002,** 78, 57-61.
(39) Mauricio, J. C.; Moreno, J.; Luis, Z.; Ortega, J. M.; Medina, M., The effects of grape must fermentation conditions on volatile alcohols and esters formed by *Saccharomyces cerevisiae*. *J. Sci. Food Agric* **1997,** 75, 155-160.
(40) Bertrand, A.; Torres-Alegre, V., Incidence de L'action de L'oxygène sur la formation des produits secondaires de la fermentation alcoolique du moût de raisin. *Science des Aliments* **1984,** 4, 45-64.
(41) Bardi, L.; Cocito, C.; Marzona, M., *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeriation and in abscence of exogenous lipids. *Int. J. Food Microbiol* **1999,** 47, 133-140.
(42) Moio, L.; Ugliano, M.; Genovese, A.; Gambuti, A.; Pessina, R.; Piombino, P., Effect of antioxidant protection of must on volatile compounds and aroma shelf life of Falanghina (Vitis vinifera L.) wine. *J. Agric. Food Chem.* **2004,** 52, 891-897.
(43) Van Wyk, C. J.; Louw, A.; Rabie, I. M. In *The effect of reductive wine making conditions on wine quality and composition*, 11th Int. Oenol. Symp. 3-5 June 1996, Sopron, Hungary, 1996; Lemperle, E.; Trogus, H.; Figlestahler, P., Eds. Sopron, Hungary, 1996; pp 180-200.
(44) Garde-Cerdán, T.; Ancín-Azpilicueta, C., Effect of SO_2 on the formation and evolution of volatile compounds in wines. *Food Control* **2007,** 18, (12), 1501-1506.
(45) Marais, J., Terpenes in the aroma of grapes and wines: A review. *S. Afr. J. Enol. Vitic.* **1983,** 4, (2), 49-60.
(46) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D., *Handbook of Enology*. 2 ed.; John Wiley & Sons Ltd: Chichester, 2006; Vol. 2.
(47) Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V., Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007,** 55, 6674-6684.
(48) Williams, P. J.; Strauss, C. R.; Wilson, B., Hydroxylated linalool derivatives as precursors of volatile monoterpenes of muscat grapes. *J. Agric. Food Chem.* **1980,** 28.
(49) Marais, J., Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *S. Afr. J. Enol. Vitic.* **1998,** 19, (1), 10-16.
(50) Versini, G.; Inama, S.; Sartori, G., A capillary column gaschromatographic research into the terpene constituents of "Riesling Romano" (Rhine Riesling) wine from Trentino Alto Adige: Their distribution within berries, their passage into the must and their presence in the wine according to different wine-making procedures. Organoleptic considerations. *Vini Ital* **1981,** XXIII, 189-211.
(51) Papadopoulou, D.; Roussis, I. G., Inhibition of the decline of linalool and α-terpineol in muscat wines by glutathione and N-acetyl-cysteine. *It. J. Food Sci* **2001,** 13, 413-419.
(52) Roussis, I. G.; Lambropoulos, I.; Tzimas, P., Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *Am. J. Enol. Vitic* **2007,** 58, (2), 274-278.
(53) Roussis, I. G.; Lambropoulos, I.; Papadopoulou, D., Inhibition of the decline of volatile esters and terpenols during oxidative storage of Muscat-white and Xinomavro-red wine by caffeic acid and N-acetyl-cysteine. *J. Agric. Food Chem.* **2005,** 93, 485-492.
(54) Louw, L. Chemical characterization of South African Young Wines. University of Stellenbsch, 2007.
(55) Piñeiro, Z.; Palma, M.; Barroso, C. G., Determination of terpenoids in wines by solid phase extraction and gas chromatography. *Anal. Chim. Acta.* **2004,** 513, 209-214.
(56) Du Toit, W. J.; Lambrechts, M. G., The enumeration and identification of acetic acid bacteria from South Africa red wine fermentations. *Int. J. Food Microbiol* **2002,** 74, 57-64.
(57) Prillinger, F.; Madner, A.; Kovcs, J., Die flüchtigen inhalsstoffe des Aepfel- und Traubensaftes. *Früchteverwertung* **1968,** 18, 98-105.
(58) Ribéreau-Gayon, P.; Dubourdieu, D.; Doneche, B.; Lonvaud, A., *Handbook of Enology*. 2 ed.; John Wiley & Sons Ltd: Chichester, 2006; Vol. 1.

Chapter 5

General discussion and conclusions

5. General discussion and conclusions

5.1 CONCLUSIONS AND FUTURE PROSPECTS

Wine is a highly complex mixture of compounds which largely define its appearance, aroma, flavour and mouth-feel properties. The aroma and flavour profile of Sauvignon blanc wine is the result of an almost infinite number of variations in production, whether in the vineyard or the winery. In the winery, the winemaker employs a variety of techniques and tools to produce wines with specific flavour profiles. One of these tools is the extent of oxygen exposure during the winemaking process and the use of SO_2.

The over all aim of this study was to investigate the effect of different O_2 and SO_2 additions to Sauvignon blanc juice. From Chapter 2 it was clear that a large amount of knowledge exists on the mechanisms and effects of oxidation in both white juice and wines. The impacting aroma factors in Sauvignon blanc wines have also been identified to a large extend. However, the transformation of these compounds between must and wine, as well as the roles O_2 and SO_2 play in this still need to be elucidated further.

In Chapter 3, it was shown that the polyphenols, especially *trans*-caftaric acid decreased when enzymatic oxidation occurred, which probably resulted in the formation of the very reactive *o*-quinone. This also caused a decrease in glutathione due to the reaction between the glutathione molecule and the formed *o*-quinone, forming the grape reaction product (Singleton *et al.*, 1985; Cheynier *et al.*, 1986). The reduction in glutathione levels was significantly reduced when sufficient SO_2 was present. Individual volatile thiols differed in their reactions to the treatments, but in general were protected against oxidation by the antioxidant function of SO_2. This was probably due to the decreased amount of *o*-quinones (due to enzymatic inhibition) available to react with the aromatic thiols. Furthermore, it seems as if the addition of oxygen to the must with the protection of SO_2 could lead to an increase in the 3SH precursor, Glut-3SH, possibly resulting in higher concentrations of 3SH and 3SHA in wines (Roland *et al.*, 2010). The presence of sufficient SO_2 thus seems to protect the juice from oxidation, even when agitated with air. When juice with a high oxidation potential (high phenolic contents and low SO_2 content) are handled, the use of inert gas could be advised to limit excessive formation of the *o*-quinone. However, the excessive use of inert gas would seem to be unnecessary, provided enough free SO_2 is present, when juice with a low oxidation potential (low phenolic content and higher free SO_2 values) is handled. This result could reduce the production costs of cellars producing Sauvignon blanc wines. However, the addition of SO_2 especially during grape pressing would thus be advisable in systems where an inert atmosphere can not be established. The concentration of methoxypyrazines, IBMP and IPMP, were not affected by the treatments (Marais, 1998). This study clearly showed the effectiveness of moderate SO_2 additions in protecting thiols from unwanted association with *o*-quinones in press juice, which could be a useful tool for manipulating Sauvignon blanc wine style.

In Chapter 4 the effect of the treatments on other volatile aroma compounds either produced metabolically or released from precursors by the yeast during fermentation were investigated. These analyses included esters, higher alcohols, fatty acids and monoterpenes. Results varied for the aroma groups and no clear trend was observed. However, it was evident that the presence of SO_2 had a significant effect on most of these compounds, regardless of whether O_2 was added or not. However, oxygen or the interaction of O_2 and SO_2 did influence individual aroma compounds in some cases. The effects of the treatments on the volatiles were not always seen in wines made from two different juices, indicating other factors such as grape composition also playing a fundamental role.

Not only is the composition of wine challenging to unravel, but consumer and market preferences will dictate the type of measurement that will be important. Nevertheless, it is a common fact that wine flavour is one of the key drivers of consumer choice. Future research should focus on winemaking processes to further improve and protect the aroma profile of Sauvignon blanc wines and other white grape varieties. For this, a greater understanding of the evolution of volatiles during fermentation and winemaking in general, is needed. These studies should especially focus on the effect of different viticultural and oenological treatments on the volatile thiol content of wines. Sensory investigations were not part of this study and should be included in future studies to determine the impact of reductive and oxidative winemaking on Sauvignon blanc wines. However, it was clear from this study that O_2 and SO_2 plays a major role in the production process of Sauvignon blanc wines and this study could have a significant impact on how this important cultivar is handled in future in the cellar.

5.2 LITERATURE CITED

Cheynier, V., Trousdale, E., Singleton, V. L., Salgeus, M. & Wylde, R., 1986. Characterization of 2-S-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. J. Agric. Food Chem. 34, 217-221.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. S. Afr. J. Enol. Vitic. 19 (1), 10-16.

Roland, A., Vialaret, J., Razungles, A., Rigou, P. & Schneider, R., 2010. Evolution of *S*-Cysteinylated and *S*-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. J. Am. Chem. Soc. 58, 4406-4413.

Singleton, V. L., Salgues, J., Zaya, J. & Trousdale, E., 1985. Caftaric acid disappearance and conversionto products of enzymatic oxidation in grape must and wine. Am. J. Enol. Vitic. 36, 50-56.